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TOXICITY OF DEGDN, SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS, SOLVENT YELLOW 33 AND SOLVENT GREEN 3 TO FRESHWATER AQUATIC ORGANISMS

FINAL REPORT FOR PHASE II

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Shady Side, MD 20764

JANUARY 1987

Supported by

U.S. Army Medical Research and Development Command
Health Effects Research Division
Fort Detrick, Frederick, MD 21701-5012

U.S. Navy Contract No. N00039-87-C-5301 via ARMY MIPR85MM5505
The Johns Hopkins University
Applied Physics Laboratory
Johns Hopkins Road
Laurel, MD 20707

Project Officer: Major John A. Kelly
Health Effects Research Division
U.S. Army Biomedical Research and
Development Laboratory
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EXECUTIVE SUMMARY

The U.S. Army Biomedical Research and Development Laboratory (USABRDL) has responsibility for assessing the possible health and environmental hazards associated with munitions-unique pollutants released during manufacturing activities and deployment in the field. The Health Effects Research Division of USABRDL has recently expressed interest in determining the acute toxicity of four munitions to freshwater aquatic organisms: Diethyleneglycol dinitrate (DEGDN), synthetic-HC (hexachloroethane) smoke combustion products, solvent yellow 33 [2-(2'-quinolinyl)-1,3-indandione], and solvent green 3 (1,4-di-p-toluidinoanthraquinone). The acute toxicity of each munition was determined for the fathead minnow (Pimephales promelas), channel catfish (Ictalurus punctatus), bluegill (Lepomis macrochirus), rainbow trout (Salmo gairdneri), water flea (Daphnia magna), amphipod (Gammarus pseudolimnaeus), midge larva (Paratanytarsus parthenogeneticus), mayfly larva (Hexagenia bilinata), and the green alga (Selenastrum capricornutum).

The toxicity of DEGDN was relatively low to the nine freshwater species tested, especially when compared to other commonly used nitrate ester explosives such as nitroglycerin and ethyleneglycol dinitrate. Toxicity values ranged from a 5-day EC50 (standing crop) of 39.1 mg/L for Selenastrum capricornutum to a 96-h LC50 of 491.4 mg/L for the Pimephales promelas. The most sensitive invertebrate tested was Daphnia magna which was more sensitive than the most sensitive fish tested, Lepomis macrochirus. Due to its high water solubility, DEGDN could cause environmental problems if sufficient amounts of the material enter an aquatic ecosystem.

The dissolved components of the synthetic-HC smoke combustion products mixture were found to be quite toxic to a number of freshwater species, especially Selenastrum capricornutum, Salmo gairdneri and Daphnia magna. A test solution containing only 5.6% of a stock mixture of these components caused both an algistatic and algicidal effect on the alga. LC50 values for the rainbow trout and the water flea were 2.2% and 9.3% of the stock solution, respectively. Additional tests with the water flea indicated that zinc was the major toxic component of the mixture. Information concerning environmental levels of the various components after use or disposal of the munitions is necessary in order to assess possible hazards to aquatic life.

Solvent yellow 33 and solvent green 3 were not toxic to seven of nine freshwater species when tested at their solubility limits. A solubility limit solution of solvent green 3 killed 50% of the rainbow trout exposed for 96 h but was non-toxic when diluted by 50%. Solvent yellow 33 was non-toxic to the rainbow trout during 96 h of exposure at its solubility limit. Both dyes caused a reduction in alga growth at solubility limits, with

solvent green 3 being most detrimental, causing a 98 - 99% reduction after 5 days of exposure. These dyes could cause a problem if released to the environment since detrimental effects were found at their solubility concentrations (i.e., 0.2 mg/L solvent yellow 33 and < 0.002 mg/L green component of solvent green 3).

This research was conducted in accordance with GLP regulations for nonclinical laboratory studies (EPA 1983. Fed. Reg. 48: 53946-53969). These studies were inspected by the JHU/APL Quality Assurance unit, and the results in the final report were audited for completeness and accuracy of reporting to the raw data.

ACKNOWLEDGEMENTS

We would like to thank Michael J. Lenkevich of The Applied Physics Laboratory for his analysis of samples during the project. We would also like to thank Major John A. Kelly and Dr. William van der Schalie of the U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, MD, for their constructive suggestions and help throughout the study. Florence H. Broski, of the same group, conducted invaluable statistical analyses of the Selenastrum capricornutum toxicity data. This research project was sponsored by the U.S. Army Medical Research and Development Command, Fort Detrick, MD, under Contract No. ARMY MIPR 85 5505 through the Applied Physics Laboratory's NAVSEA Contract No. N00024-83-C-5301.

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SECTION 1

INTRODUCTION

1.1 General

The U.S. Army Biomedical Research and Development Laboratory (USABRDL) has responsibility for assessing the possible health and environmental hazards associated with munitions-unique pollutants released during manufacturing activities and deployment in the field. The Health Effects Research Division of USABRDL has recently expressed interest in determining the acute toxicity of the explosive propellant Diethyleneglycol dinitrate (DEGDN) and three smoke munitions compounds: synthetic-HC (hexachloroethane) smoke combustion products, solvent yellow 33 [2-(2'-quinolinyl)-1,3-indandione], and solvent green 3 (1,4-di-p-toluidinoanthraquinone) to freshwater aquatic organisms. The acute toxicity of each munition was determined for the fathead minnow (Pimephales promelas), channel catfish (Ictalurus punctatus), bluegill (Lepomis macrochirus), rainbow trout (Salmo gairdneri), water flea (Daphnia magna), amphipod (Gammarus pseudolimnaeus), midge larva (Paratanytarsus parthenogeneticus), mayfly larva (Hexagenia bilinata), and the green alga (Selenastrum capricornutum).

1.2 DEGDN

DEGDN is an explosive propellant chosen for use as a plasticizer replacement for nitroglycerin in the propellant mixtures for the 120 mm shell of the M1 Abrams tank. In the past it has been manufactured and processed at Radford Army Ammunition Plant, Radford, VA, which discharges waste effluents to the New River. It is presently manufactured at the Naval Ordinance Station, Indian Head, MD, which discharges waste effluents to the Potomac River. The potential for release of DEGDN into streams and rivers means that some information on its toxicity to aquatic organisms is needed to allow reasonable discharge standards to be established by regulatory authorities. The objective of this study was to determine the acute toxicity of dissolved DEGDN to nine freshwater species.

The solubility of DEGDN in water has been shown to be 0.4 g per 100g at 20° C, indicating the potential of high aqueous concentrations in streams subject to DEGDN discharge (Linder 1980). Environmental fate studies indicate that DEGDN is a stable compound once dissolved in water (photolytic half-life of 29 to 40 days; hydrolytic half-life of 144 days) with a low K_{ow} of 9.6 and a low affinity for absorption to sediment (Spanggord et al. 1985). This same study indicated that microbial biotransformation will be a major fate process for DEGDN when it is introduced into waters with other organic waste substrates.

DEGDN toxicity data are sparse. Krasovsky et al. (1973) reported LD50s for rats of 777 mg/kg (oral) and for guinea pigs

and white mice of 1250 mg/kg (peroral in vegetable oil), indicating that it is less toxic than nitroglycerin or ethyleneglycol dinitrate, two other commonly used nitrate ester explosives. They set a maximum permissible level for DEGDN in reservoir waters of 1.0 mg/L based on human perception thresholds and influences on biochemical oxygen uptake, mineralization of organic impurities and dynamics of growth of saprophytic microflora. Holleman et al. (1983) stressed a need for aquatic toxicity studies on DEGDN and other nitrate esters but suggest that DEGDN should be less toxic than nitroglycerin due to its increased molecular weight. Bentley et al. (1978) found nitroglycerin to be acutely toxic to freshwater aquatic organisms in amounts ranging from 1.67 mg/L (96-h LC50 for bluegill) to 55 mg/L (48-h EC50 for Chironomus tentans).

1.3 Synthetic-HC Smoke Combustion Products Mixture

Synthetic-HC smoke combustion products are the combustion products of the M8 grenade, M5 smoke pot and M4A1 floating smoke pot. The percent composition, by weight, of this mixture is given in Table 1. These percentages were derived by the Army from published reports (Katz et al. 1980, Spanggord et al. 1985). All but aluminum and hexachloroethane are considered priority pollutants by EPA (USEPA 1986a). These products represent a complex chlorinated organic/metallic mixture dominated by zinc. When the above devices are used in training exercises, these components are released to the environment where they could damage aquatic life. Also, there is a considerable stockpile of these munitions on hand. In the event this stockpile has to be discarded, the question of its potential effect on aquatic organisms needed to be quantified.

Data for the prediction of the toxicity of this mixture are not available. There is data concerning the toxicity of the individual components to aquatic organisms, since most are priority pollutants. For example, acute toxicity values for zinc range from 50.6 µg/L for Ceriodaphnia reticulata to 86.7 mg/L for a damselfly (USEPA 1986b). This range covers 43 species of freshwater organisms. Toxicity values for the parent compound of this mixture, hexchloroethane, range from a 96-h LC50 of 0.98 mg/L for rainbow trout and bluegill to a 48-h EC50 of 8.07 mg/L for the water flea (USEPA 1980b).

The objectives of this study were twofold: first, to determine the acute toxicity of the total mixture of dissolved components to freshwater organisms; second, to determine the major toxic component(s) of the mixture using a series of static tests with the water flea

1.4 Solvent Yellow 33 and Solvent Green 3

Solvent yellow 33 and solvent green 3 are components of a smoke munition. The munition contains 42% by weight of the dyes. Solvent green 3 is a 30:70 mixture of solvent yellow 33 [2-(2'-

TABLE 1. PERCENT COMPOSITION, BY WEIGHT, OF THE SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE

Hydrochloric acid (HCl)	1.828
Carbontetrachloride (CCl_4)	5.515
Tetrachloroethylene (C_2Cl_4)	14.411
Hexachloroethane (C_2Cl_6)	4.480
Hexachlorobenzene (C_6Cl_6)	1.634
Aluminum oxide (Al_2O_3)	10.917
Zinc chloride ($ZnCl_2$)	61.073
Lead chloride ($PbCl_2$)	0.089
Cadmium chloride ($CdCl_2$)	0.051
Arsenic chloride ($AsCl_3$)	0.001

quinolinyl)-1,3-indandione] and a green dye component (1,4-di-p-toluidinoanthraquinone). Data concerning the acute toxicity of these dyes to aquatic organisms are not available. Solvent yellow 33 has been shown to cause hypersensitivity reactions in humans exposed to 10 µg/g in insult patch testing (Weaver 1983). Research is presently underway to determine the inhalation toxicity of these dyes (Henderson et al. 1984). The objective of this study was to determine the toxicity of these dyes to nine freshwater species as a necessary data base for establishing water quality criteria.

SECTION 2

OBJECTIVES OF STUDY

1) To determine the acute toxicity of DEGDN, synthetic-HC smoke combustion products mixture, solvent yellow 33 and solvent green 3 to nine species of freshwater aquatic organisms.

2) To determine the major toxic component(s) of the synthetic-HC smoke combustion products mixture to Daphnia magna.

SECTION 3

MATERIALS AND METHODS

3.1 Toxicant Characterization and Analytical Chemistry

3.1.1 DEGDN

The propellant was obtained from the Naval Ordinance Station (NOS), Indian Head, MD. Upon manufacture, the pure DEGDN was dissolved in absolute ethanol for shipment. When dissolved in ethanol, the propellant can be shipped as a flammable liquid rather than an explosive. The DEGDN received was 2.86% by weight in ethanol. HPLC analysis of the DEGDN indicated no contaminants in this stock solution (Fisher et al. 1985). The liquid was stored at room temperature in the dark since DEGDN becomes less stable at lower temperatures.

Analysis of aqueous DEGDN samples was done by HPLC (Waters μBONDAPAC C_{18} column) using an isocratic 30% water : 70% methanol mobile phase and a detector wavelength of 215 nm. Aqueous samples were injected directly into the HPLC after filtration to 0.45 μm. This analytical technique allowed for a detection limit of 0.286 mg/L. Fisher et al. (1985) showed no loss of the compound in dilution water over 48 h at 22°C. The details of the analytical technique, including precision and accuracy data along with solubility and stability information are described in Fisher et al. (1985). Holleman et al. (1983) describe the chemical and physical properties of this munition.

3.1.2 Synthetic-HC Smoke Combustion Products Mixture

Synthetic-HC smoke combustion products are a complex mixture whose composition is given in Table 1. The stock test solution was mixed prior to each test using these percentages. In this way, we avoided combusting munitions and collecting individual components for the tests. HPLC grade solvents were used for the liquid chlorinated organics, while ultrapure HCl and metallic compounds were used for the inorganic portions of the mix. These components were added to diluent well water and allowed to mix and settle for 24 and 6 h, respectively.

The chlorinated organics analytical protocol was a modification of Standard Method 509A (APHA et al. 1985) for the analysis of organic pesticides. A pentane extraction/concentration (10X) procedure was used to remove the chlorinated organics from the water prior to injection into a gas chromatograph equipped with an electron-capture detector. Aluminum, lead, cadmium and arsenic were analyzed by EPA's atomic absorption(AA) spectrophotometric graphite furnace Methods 202.2, 239.2, 213.2 and 206.2, respectively (USEPA 1983). Arsenic was also analyzed by EPA's AA spectrophotometric gaseous hydride Method 206.3. The zinc was analyzed by EPA's direct aspiration flame AA spectrophotometric Method 289.1. The details of the

analytical technique, including precision and accuracy data along with individual compound solubility and stability, are given in Fisher et al. (1985).

The analytical methods allowed for detection limits for the individual components of: 0.01 mg/L - chlorinated organics (0.005 mg/L with increased injection volume), 0.002 mg/L - Al, 0.0002 mg/L - Cd, Pb and As and 0.00008 mg/L - Zn. Due to the large number of individual components in the mixture, results of the solubility and stability experiments from Fisher et al. (1985) will not be detailed here. Concentrations of components in the 100% stock mixture ranged from 0.0015 mg/L for As to 258 mg/L for Zn (Table 10 and Appendix A). Stability of the components in the stock mixture varied with component, time and temperature.

3.1.3 Solvent Yellow 33 and Solvent Green 3

Technical grade and purified solvent yellow 33 and solvent green 3 were received from the Inhalation Toxicology Research Institute (ITRI), Lovelace Biomedical and Environmental Research Institute, Albuquerque, NM. Chemically, the technical grade dye materials used in testing were 93 - 95% pure, with the major contaminants being the precursors used in synthesis or, in the case of solvent yellow 33, an apparent artifact of the synthesis process in which 3 instead of 2 molecules combined (Henderson et al. 1984).

Aqueous samples were analyzed by HPLC (Waters μBONDAPAC C_{18} column) using an isocratic 10% water : 90% methanol mobile phase for solvent yellow 33 and a linear gradient from 10% water : 90% methanol to pure methanol for solvent green 3. The UV detector wavelength for both dyes was 254 nm. The details of the analytical technique, including precision and accuracy data along with solubility and stability information are described in Fisher et al. (1985). Aqueous samples were injected directly into the HPLC following filtration to 0.22 μm. This analytical method allowed a detection limit of 0.08 mg/L for both dyes. Use of a C_{18} solid phase extraction cartridge allowed for an increase in sensitivity to 0.002 mg/L. Initial studies on the solubility of these dyes indicated that the solubility of solvent yellow 33 ranged from 0.09 mg/L at 12°C to 0.17 mg/L at 22°C. The yellow 33 component of solvent green 3 exhibited the same solubility, although the green component of this mixture was never detectable, even with a detection limit of 0.002 mg/L. Both dyes were stable once dissolved in water, with no loss of compound in a 48 h period (Fisher et al. 1985).

3.2 Test Solution Preparation

3.2.1 DEGDN

Test solutions of DEGDN for the fish and invertebrate tests were prepared by vacuum evaporation of the ethanol from the

shipping solution and dissolving the DEGDN in diluent well water. This solution was then brought to the proper test temperature and used as the stock. The stock solution varied in DEGDN concentration from 600 mg/L for the invertebrates to 3500 mg/L for the fish. Appropriate dilutions of these stocks were made with diluent water to achieve the range of test concentrations necessary. The stock solutions and individual test solutions were analyzed for aqueous DEGDN concentration. For the Selenastrum capricornutum bioassay, the pure DEGDN was dissolved in the algae medium described later, and dilutions were made with this same medium.

3.2.2 Synthetic-HC Smoke Combustion Products Mixture

The water-soluble fractions of the synthetic-HC smoke combustion products were obtained with a method similar to that used by Anderson et al. (1974) to determine the water-soluble fractions of crude and refined oils. Diluent water (15.5 L) was placed in a 22.5 L pyrex jar and brought to the appropriate test temperature in an incubator (i.e., 24, 22, 17, or 12°C). For the alga test, 15.5 L of algal media was used instead of diluent freshwater. The chlorinated organics, metals and HCl were added in the proportions shown in Table 2. Range-finding tests indicated that dilutions of this stock mixture would be toxic and allow for determinations of LC50 values. A stir bar was added, and the jar was sealed and placed on a magnetic stir plate in an incubator at the appropriate temperature. The stirring speed was adjusted to create a vortex which extended to approximately 25% of the water depth. After 24 h of stirring, the jar was allowed to stand for 6 h to allow for settling of particulates. Appropriate dilutions were made from this 100% stock solution and used to determine LC50 values based on percentage dilutions as in an effluent test.

Water samples were taken from the stock to determine individual component concentrations for each test. For tests with Daphnia magna and Salmo gairdneri, samples of each test dilution were analyzed at the beginning and end of each static renewal test period and at the beginning and end of the static tests. A new 100% stock solution was prepared for each renewal.

Further tests were conducted to determine the major toxic component(s) of this mixture to Daphnia magna. The following component mixes were tested: total mix, metals only, organics only, Zn and HCl only and the total mix less Zn. Each component mix was prepared in the same manner as the total stock mixture described above. Each component was added in the amount shown in Table 2. LC50 values were based on the percentage dilution of the stock component mix. For the Zn plus HCl tests, LC50 values were also based on measured Zn. All the stock mixes and dilutions were analyzed for all individual components present.

TABLE 2. QUANTITIES OF COMPONENTS OF THE SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE ADDED TO 15.5 L DILUENT WATER DURING TOXICITY STUDIES

Component	Percent of Total	Amount Added	Concentration if All Dissolved (mg/L)
HCl	1.828	28.41 mg	1.83
CCl_4	5.515	85.06 mg	5.49
C_2Cl_4	14.411	223.68 mg	14.43
C_2Cl_6	4.480	69.57 mg	4.49
C_6Cl_6	1.634	25.40 mg	1.64
Al_2O_3	10.917	169.30 mg	5.78 (Al)
$ZnCl_2$	61.073	946.63 mg	29.39 (Zn)
$PbCl_2$	0.089	1.38 mg	0.066 (Pb)
$CdCl_2$	0.051	0.79 mg	0.031 (Cd)
$AsCl_3$	0.001	0.02 mg	0.0005(As)
	Total	1,545.70 mg	

3.2.3 Solvent Yellow 33 and Solvent Green 3

Technical grade formulations of the dyes were added to aerated diluent water or filter sterilized algal media and stirred for 24 h at the appropriate test temperature. Excess amounts were added in order to achieve a saturated solution of each dye (Fisher et al. 1985). The stock solutions were vacuum filtered to 0.22 µm and used to make appropriate test dilutions. Aqueous concentrations of the dyes in the stock solutions and dilutions were determined so that LC50 values could be based on measured dye concentrations.

3.3 Dilution Water Quality

The dilution water used in the fish and invertebrate tests was obtained from a non-chlorinated deep well. A comprehensive analysis of this well water is provided in Table 3.

3.4 Test Organisms

Specific information on the organisms used in testing is given in Table 4. The diurnal photoperiod for the fish and invertebrates during culture and holding was 16 h light: 8 h dark. Lighting was provided by normal laboratory fluorescent lights of 50-100 foot candles intensity. During holding, the rainbow trout were fed trout food pellets of appropriate size supplied by the National Fisheries Center - Leetown, WV. The other fish were fed live Artemia until they were large enough to take flake food. Rainbow trout, bluegill and catfish were obtained from fish hatcheries, held for two weeks and tested. Fathead minnow stock cultures were maintained in 76 L aquaria at 25°C (± 2°C) using a recirculating system. Adult fish were fed flake food once each day ad libitum. Spawning substrates consisted of inverted 15-cm sections of 10-cm diameter clay tile or PVC pipe cut in half longitudinally. Eggs were collected from the substrates and hatched in a separatory funnel under vigorous aeration. Upon hatching, larvae were transferred to a rearing tank and fed freshly-hatched Artemia less than 24 h old. The larvae were maintained for 2 weeks at 22°C (± 1°C) before testing.

A starter culture of Daphnia magna was obtained from the National Fisheries Research Laboratory, Columbia, MO. This culture was used to initiate a continuous in-house culture. Twenty adult daphnids were stocked into each of seven 2.5 L culture tanks. Aerated well water flowed through each tank at a rate of 20 mL/min. Daphnids were fed Selenastrum capricornutum daily ad libitum. Cultures were maintained at 22°C (± 1°C). Neonates were removed 3 times/week or 24 h prior to the initiation of a test.

Hexagenia bilinata nymphs were acquired from Robinson Wholesalers of Lake Geneva, WI. The nymphs, which were approximately 2-5 cm total length, were stocked at densities of

TABLE 3. COMPREHENSIVE DILUTION WATER ANALYSIS, 1986

Base/neutrals

Compound	ug/L[a]	Compound	ug/L[a]
N-Nitrosodimethylamine		Di-n-butylphthalate	
bis(2-Chloroethyl)Ether		Fluoranthene	
1,3-Dichlorobenzene		Benzidine	
1,4-Dichlorobenzene		Pyrene	
1,2-Dichlorobenzene		Butylbenzylphthalate	
bis(2-Chloroisopropyl)Ether		3,3'-Dichlorobenzidine	
N-Nitroso-di-n-propylamine		Benzo(a)Anthracene	
Hexachloroethane		bis(2-Ethylhexyl)phthalate	
Nitrobenzene		Chrysene	
Isophorone		Di-n-octylphthalate	
bis(2-Chloroethoxy)Methane		Benzo(b)Fluoranthene	
1,2,4-Trichlorobenzene		Benzo(k)Fluoranthene	
Naphthalene		Benzo(a)Pyrene	
Hexachlorobutadiene		Indeno(1,2,3-cd)Pyrene	
Hexachlorocyclopentadiene		Dibenzo(a,h)Anthracene	
2-Chloronaphthalene		Benzo(g,h,i)Perylene	
Dimethylphthalate			
Acenaphthylene		The following are non-priority pollutant hazardous substance list compounds.	
Acenaphthene			
2,4-Dinitrotoluene			
2,6-Dinitrotoluene		Aniline	
Diethylphthalate		Benzyl Alcohol	
4-Chlorophenyl-phenylether		4-Chloroaniline	
Fluorene		2-Methylnaphthalene	
N-Nitrosodiphenylamine		2-Nitroaniline	
1,2-Diphenylhydrazine		3-Nitroaniline	
4-Bromophenyl-phenylether		Dibenzofuran	
Hexachlorobenzene		4-Nitroaniline	
Phenanthrene			
Anthracene		DETECTION LIMIT	1

(Continued next page)

TABLE 3. (CONTINUED)

Pesticides		Metals	
Compound	ug/L[a]	Metal (Total)	mg/L
alpha-BHC		Aluminum	<0.10
beta-BHC		Arsenic	<0.002
delta-BHC		Calcium	50
gamma-BHC (Lindane)		Chromium	<0.003
Heptachlor		Copper	0.002
Aldrin		Mercury	<0.0002
Heptachlor Epoxide		Lead	<0.003
alpha-Endosulfan		Selenium	<0.003
Dieldrin		Tin	<0.004
4,4'-DDE		Zinc	<0.05
Endrin		Iron	1.7
beta-Endosulfan			
4,4'-DDD		Water Quality	
Endrin Aldehyde			
Endosulfan Sulfate		pH	7.8
4,4'-DDT		Alkalinity	156 mg/L as $CaCO_3$
Methoxychlor			
Chlordane		Hardness	190 mg/L as $CaCO_3$
Toxaphene			
Aroclor 1016			
Aroclor 1221			
Aroclor 1232			
Aroclor 1242			
Aroclor 1248			
Aroclor 1254			
Aroclor 1260			
Kepone			
DETECTION LIMIT	0.05		

[a]Concentrations less than the detection limit are left blank.

TABLE 4. INFORMATION ON ORGANISMS USED IN TESTING

Species	Original Source[a]	Mean Size used in Testing Length(mm)	Weight(mg)	Age (weeks)
FISH				
Fathead Minnow (Pimephales promelas)	Newton Fish Hatchery Cincinnati, OH	10.3	8.0	2
Channel Catfish (Ictalurus punctatus)	Kurtz Fish Hatchery Elverson, PA	14.2	30.1	2
Bluegill (Lepomis macrochirus)	Kurtz Fish Hatchery Elverson, PA	18.3	108	4
Rainbow Trout (Salmo gairdneri)	National Fisheries Ctr. Kearneysville, WV	23.8	149	3
INVERTEBRATES				
Water flea (Daphnia magna)	National Fisheries Research Lab Columbia, MO	NA[b]	NA	<24 hrs
Midge Larva (Paratanytarsus parthenogeneticus)	USEPA - ERL Duluth, MN	NA	NA	2nd-3rd Instar
Mayfly Larva (Hexagenia bilinata)	Robertson Wholesalers Lake Geneva, WI	NA	NA	Late Instar
Amphipod (Gammarus pseudolimnaeus)	Little Plover River Stevens Point, WI	NA	NA	Early Young
ALGAE				
Selenastrum capricornutum	Star Culture Collection N. Texas State Univ. Denton, TX	NA	NA	NA

(Continued next page)

TABLE 4. (CONTINUED)

Species	Acclimation (weeks)	Loading in Tests (g/L test solution)	Holding and Testing Temperature (°C)	Prior Disease Treatments
FISH				
Fathead Minnow (*Pimephales promelas*)	2	0.230	22 ± 2	None
Channel Catfish (*Ictalurus punctatus*)	2	0.115	22 ± 2	None
Bluegill (*Lepomis macrochirus*)	2	0.415	22 ± 2	None
Rainbow Trout (*Salmo gairdneri*)	2	0.497	12 ± 2	None
INVERTEBRATES				
Water flea (*Daphnia magna*)	NA	NA	22 ± 2	NA
Midge Larva (*Paratanytarsus parthenogeneticus*)	NA	NA	22 ± 2	NA
Mayfly Larva (*Hexagenia bilinata*)	NA	NA	22 ± 2	NA
Amphipod (*Gammarus pseudolimnaeus*)	NA	NA	17 ± 2	NA
ALGAE				
Selenastrum capricornutum	NA	NA	24 ± 2	NA

[a]All test organisms obtained from in-house cultures except channel catfish, bluegill and rainbow trout.

[b]NA = not applicable.

500/200 L culture tank. These tanks contained 100 L of aerated well water and 4-5 cm of autoclaved biocide-free garden soil. Agricultural lime and composted hay were added to the substate to raise the pH to a range of 7-8 and increase organic material to approximately 15%. Temperature was raised from 5°C to 22°C over a period of 9 days. Thereafter, temperature was maintained at 22°C (± 1°C). They were fed Tetra-min at a rate of 15 g/tank twice/week until testing began.

Eggs of Paratanytarsus parthenogeneticus were obtained from the USEPA Environmental Research Laboratory in Duluth, MN. Eggs were stocked at a density of 250/40 L tank. Each tank contained 7 L of aerated well water maintained at 22°C (± 1°C). After hatching, larvae were fed a trout chow/cerophyll suspension as needed to maintain a supply of food in the bottom of each tank. Upon emergence, adults were aspirated into a 500 mL Erlynmeyer flask containing 75 mL of aerated well water and allowed to oviposite overnight. Egg masses were collected the following morning and the process was repeated. Larvae used for testing purposes were collected from 7 day old cultures by dislodging them from their tubes with a gentle stream of water.

Gammarus pseudolimnaeus were collected from the Little Clover River, Portage County, WS. They were shipped to APL and stocked at densities of 70 per 40 L culture tanks containing 30 L of aerated well water. The culture tank substrate consisted of an assortment of aged leaves collected from local streams. Cultures were maintained at 17°C (± 1°C). Aerated well water was used to replace 1/2 of the water in each tank three times/week. The amphipods were fed at a rate of 0.2 g/tank twice/week with Tetra-min. Young for tests were removed from the tanks using a 4 mm diameter pipette.

Selenastrum capricornutum starter culture was purchased from North Texas State University, Denton, TX. Stock algal cultures were reared in 2.5 L Pyrex culture flasks containing 1 L of sterilized assay medium prepared according to Miller et al. (1978). Cultures were maintained under constant cool-white fluorescent lights (4300 lux ± 10%) at a temperature of 24°C (± 1°C) on a shaker table oscillating at 100 rpm (± 10%). Algal cells for testing purposes were obtained from 8 day old stock cultures.

3.5 Test Methodologies

3.5.1 General

Static and static renewal acute bioassay methods were those recommended by The American Society for Testing and Materials (ASTM 1980). Fish used in testing were acclimated to the well water for periods indicated in Table 4. Fish were not used in testing if they had any symptoms of disease within 10 days of the start of the test, or if more than 3% died within 48 h preceding the start of the test. Feeding was discontinued 24 h prior to

the start of tests. All fish and tank assignments were random. Two replicates were used for each treatment with 10 fish per replicate. Fathead minnows were transferred to the test beakers using a fire-polished pipet, while other fish were transferred in small beakers.

Test chambers and solution volumes used in testing all organisms are given in Table 5. Temperatures were held within 1°C of the holding temperature (Table 4) by maintaining the test vessels in a constant temperature water bath. Temperature in the water bath was monitored with a probe and chart recorder. Lighting was of the same quality and photoperiod as used during holding for all species. The pH and dissolved oxygen concentrations were measured daily in one replicate of each treatment which contained live organisms. Total hardness was measured in the well water at the beginning of each test. The total duration of the fish static acute tests was 96 h. Test concentrations were renewed every 24 h where a static renewal method was used.

Invertebrate static and static renewal acute tests were similar to the fish tests except that the duration was 48 h. During the 24 h neonate collection period for daphnids, algal food was provided. Neonates were transferred to a beaker containing clean diluent water prior to the test. Other invertebrates were transferred to a common pool 24 h prior to testing and were not fed, except for Paratanytarsus parthenogeneticus, which were allowed a small amount of food to build tubes. Hexagenia bilinata were tested using small glass tubes of various diameters as substrates. Transfer of all invertebrates was done using a 2 mm fire-polished pipet. Replicate beakers were used for each treatment, with 10 organisms per beaker.

Algal toxicity test methods were based on those recommended by Payne and Hall (1979). Stock solutions were made by using filtered, sterilized assay media (Miller et al. 1978) instead of diluent well water. Test solutions were prepared by dilution of the stock solutions with uncontaminated filter sterilized assay media within a sterile transfer room. Test solutions were dispensed into Delong flasks and innoculated with Selenastrum capricornutum cells in log growth to achieve a density of 5000 cells/mL. Triplicates were prepared for each treatment. Flasks were then capped and placed onto a shaker table in an incubator set at the culturing conditions described earlier. Growth measurements (biomass and cell density) were made at 0, 24, 48, 72, 96, and 120 h. Two 5 mL algal samples were frozen and, at a later time, biomass was indirectly measured by in vivo chlorophyll a fluorescence with a Turner Designs Model 10 Fluorometer. Algal cell density was determined from a separate 5 mL sample with a Coulter Counter. Background fluorescence and cell density of a solution of AAP with the maximum amount of toxicant added was also determined. After 120 h, all treatments with mean cell counts of ≤ 6,500 cells/mL were restarted in

TABLE 5. TEST CHAMBER AND TEST SOLUTION VOLUMES

Species	Test Chamber	Test Solution Volume(L)
Fathead minnow	0.4 L Beaker	0.35
Channel catfish	3.0 L Tank	2.75
Bluegill	3.0 L Tank	2.75
Rainbow trout	3.0 L Tank	3.00
Water flea	0.4 L Beaker	0.35
Midge larva	0.4 L Beaker	0.35
Mayfly larva	3.0 L Tank	1.50
Amphipod	0.4 L Beaker	0.35
Alga	0.28 L Delong flask	0.17

uncontaminated media and allowed to grow for an additional nine days, at which time final growth measurements were recorded.

3.5.2 DEGDN

Initial tests with DEGDN indicated it to be very stable once dissolved in diluent water, therefore, all acute toxicity tests were static, using test vessels with non-airtight covers. Aqueous DEGDN samples were analyzed at the beginning and end of each test for all treatments. This compound did exert a significant oxygen demand during tests with fish. Aeration was used in treatments where dissolved oxygen approached 40% of saturation. Aeration had no effect on aqueous DEGDN concentrations. For example, in one aerated treatment vessel, DEGDN values averaged 178.9 mg/L at the start and 177.7 mg/L at the end of the test.

3.5.3 Synthetic-HC Smoke Combustion Products Mixture

Initially, both static and static renewal definitive tests were to be conducted on all test species. Midway through the study, tests with the water flea and rainbow trout showed that no difference in results occurred between the two methods. Therefore, the static method was used to test the remaining species. A full chemical analysis of all components was conducted on the 100% stock solutions and individual treatments at the beginning and end of each renewal period and the beginning and end of the static tests for the daphnids and rainbow trout. These two species were found to be the most sensitive non-algal species. For the fathead minnows, midge larvae and mayfly larvae, chemical analyses were conducted on the 100% stock solutions at each renewal. For the bluegill, catfish, amphipod and alga bioassays, full chemical analyses were conducted on the 100% stock solutions and all treatments at the beginning of each static test. All tests were conducted without aeration in closed, but not airtight, chambers.

Further tests were conducted using the various components of this mixture to determine the toxic component(s) to daphnids. These were 48 h static acute tests with chemical analyses for individual components of the 100% stock solutions and each treatment at the start of the test.

3.5.4 Solvent Yellow 33 and Solvent Green 3

Initial studies with these dyes indicated both to be minimally soluble in water but stable once dissolved (Fisher et al. 1985). Two range finding tests with Daphnia magna and Paratanytarsus parthenogeneticus indicated no toxicity from either dye at its solubility limit. Therefore, only the aqueous solubility limit was used in the remaining toxicity tests, except where toxicity was found. All tests were conducted using a static acute protocol with covered, but not air-tight chambers.

Water samples were analyzed for each dye at the beginning and end of each test in all treatments.

3.6 Test End Points and Data Analyses

In the tests with DEGDN and the dyes, LC50s were calculated based on average measured concentrations. In the tests with the synthetic-HC smoke combustion products mixture, LC50s were based on percentages of the 100% stock solutions used in the treatments rather than on individual concentrations of components, (as in an effluent toxicity test). In some instances, LC50s were also calculated based on the concentration of an individual component (i.e., zinc in the Zn only tests with daphnids).

A fish was considered dead when ventilatory movements ceased and the fish failed to respond to gentle prodding. Dead fish were removed from test chambers twice/day, at which time mortalities were recorded. All invertebrates were similarly recorded dead when they failed to show any movement following gentle stimulation. For the smaller organisms (i.e., Daphnia magna and Paratanytarsus parthenogeneticus), a microscope was used to identify dead organisms.

LC50s and their 95% fiducial limits were determined using the probit method contained in a computer program developed by Stephan (1978), unless otherwise noted. In all cases, the goodness of fit probability of the data to the probit model was greater than 0.05.

In the algal toxicity test, the end point monitored was growth, measured as density (cells/mL) and biomass (chlorophyll a in µg/L). A log(10) transformation was used for all growth measurements. When treatment groups were compared to controls, a Student's t test with Bonferroni's correction for simultaneous comparisons was used. An overall significance level of 0.05 was used in all analyses. When possible, three specific end points were evaluated:

1. Algistatic concentration. The minimum algistatic concentration was determined by an inverse regression technique described by Payne and Hall (1979) and Sokal and Rohlf (1981). In this study, an algistatic effect was said to have occurred if, after the 5-day growth period, cell counts did not increase significantly from the initial inoculum level.

2. Algicidal concentration. This is the lowest concentration tested which causes an apparent algistatic effect after 5 days and which prevents cells transferred to clean media from resuming logarithmic growth.

3. 5-day EC50 based on standing crop. Where possible, the algal bioassay data were analyzed to calculate the 5-day EC50 and its 95% confidence limits. A linear model with treatment and dry

weight (density) both transformed to log(10) was fitted to the data. Analysis of variance(ANOVA) was performed to test for fit to the model and for effects. Regression analysis was performed to provide estimated values for the day 5 data and for input used to calculate the 5-day EC50 and its associated 95% confidence limits. SAS PROC GLM computer software was used for these analyses (SAS 1982). The 5-day EC50 and its associated 95% confidence limits were computed using the FORTRAN CONFINT computer program (Feder 1981).

SECTION 4

RESULTS AND DISCUSSION

4.1 Water Quality

Dissolved oxygen varied between tests, with the lower values recorded for the larger fish species (Table 6). The lowest dissolved oxygen values were approximately 4.0 mg/L at the end of the 96-h static DEGDN and dye tests with bluegill. These values were above the required 40% saturation limit for warm water species at the test temperature (3.5 mg/L), but loading was near the limit for the test vessels. As noted earlier, aeration did not decrease the stability of the DEGDN. Values for pH were relatively constant between tests, ranging from an average of 7.3 to 8.4 at the end of the test (Table 6).

4.2 DEGDN

DEGDN proved to be relatively non-toxic to the freshwater organisms tested (Table 7). The invertebrate 48-h LC50s ranged from 90.1 to 355.3 mg/L, with the water flea being most sensitive. All the fish species had similar sensitivities (mean 96-h LC50 = 273.5 mg/L), except for the fathead minnows, which were somewhat more tolerant with an LC50 of 491.4 mg/L.

There was a dose-response relationship between Selenastrum capricornutum growth and increased DEGDN concentration (Tables 8 and 9; Figures 1 and 2). By day 5, the lowest concentration of DEGDN tested (58.4 mg/L) caused a significant reduction in algal cell density and chlorophyll a when compared to control values (Student's t tests with Bonferroni's correction for simultaneous comparisons, $p = 0.0001$). The 5-day minimum algistatic concentration based on cell density was 171.6 mg/L (95% asymmetrical confidence limits = 86.9 - 335.5 mg/L). The minimum algicidal concentration was greater than 542.4 mg/L, the highest concentration tested. When algal cultures from this treatment were restarted in clean media, they resumed logarithmic growth (Figures 1 and 2). The 5-day EC50 for DEGDN and this alga was 39.1 mg/L based on dry weight (95% asymmetrical confidence limits = 28.8 - 52.9 mg/L). Thus, Selenastrum capricornutum was the most sensitive species tested with this compound.

There are no other aquatic toxicity data available for DEGDN. Krasovsky et al. (1973) reported LD50s for rats of 777 mg/kg and for guinea pigs and white mice of 1250 mg/kg, indicating this compound to be relatively non-toxic compared to nitroglycerin and ethyleneglycol dinitrate, two similar explosive compounds. Holleman et al. (1983) cited the lack of aquatic toxicology data for DEGDN but suggested it should be less toxic than nitroglycerin due to its greater molecular weight.

Data from the present study indicates that DEGDN is less toxic than nitroglycerin. Bentley et al. (1978) found

TABLE 6. WATER QUALITY DATA FOR TOXICITY TESTS (MEAN VALUES ± S.D.)

Compound	Species	Test Type[a]	Total Hardness (mg/L $CaCO_3$)	DO (mg/L)		pH	
				Start	End	Start	End
DEGDN	Water flea	S	210	8.1(0.17)	7.6(0.28)	8.1(0.17)	8.1(0.15)
	Midge larva	S	210	7.8(0.32)	7.1(0.31)	8.0(0.10)	8.0(0.11)
	Mayfly larva	S	210	8.6(0.05)	8.1(0.50)	8.2(0.05)	7.9(0.11)
	Fathead minnow	S	210	8.6(0.05)	8.3(0.08)	8.2(0.05)	8.0(0.12)
	Rainbow trout	S	205	9.2(0.40)	7.7(0.12)	7.8(0.09)	7.6(0.17)
	Amphipod	S	192	9.0(0.05)	8.5(0.07)	8.2(0.12)	8.2(0.08)
	Bluegill	S	192	8.7(0.04)	4.1(0.18)	7.9(0.08)	7.9(0.18)
	Catfish	S	185	8.5(0.17)	5.8(1.25)	7.9(0.11)	7.3(0.15)
Synthetic-HC (total mix)							
	Water flea	S	215	8.7(0.09)	7.9(0.33)	7.6(0.10)	8.0(0.12)
		SR	215	8.7(0.09)	8.2(0.31)	7.6(0.10)	7.9(0.09)
	Midge larva	SR	215	7.3(0.52)	8.2(0.49)	7.7(0.26)	7.6(0.13)
	Mayfly larva	SR	210	8.4(0.21)	7.0(0.67)	7.7(0.25)	7.5(0.21)
	Fathead minnow	SR	210	8.4(0.21)	7.9(0.74)	7.7(0.25)	7.6(0.12)
	Rainbow trout	S	205	9.0(0.27)	7.7(0.13)	7.7(0.09)	7.7(0.16)
		SR	205	9.0(0.27)	8.5(0.28)	7.7(0.09)	7.7(0.16)
	Amphipod	S	192	8.3(0.10)	7.8(0.09)	7.6(0.37)	7.9(0.15)
	Bluegill	S	192	8.6(0.20)	4.0(0.20)	7.2(0.41)	7.4(0.18)
	Catfish	S	185	8.5(0.17)	7.5(0.18)	7.3(0.40)	7.4(0.06)
Synthetic-HC (Component toxicity)							
	Water flea						
	- Total mix	S	190	8.5(0.08)	8.2(0.12)	8.1(0.17)	8.3(0.13)
		S	190	8.6(0.05)	8.2(0.09)	8.0(0.21)	8.3(0.11)
	- Mix minus Zn	S	195	8.4(0.05)	8.0(0.05)	7.4(0.27)	7.9(0.05)
	- Metals only	S	190	8.4(0.04)	8.3(0.27)	7.2(0.29)	8.1(0.16)
	- Organics only	S	190	8.4(0.10)	8.2(0.25)	8.1(0.08)	8.4(0.05)
	- Zinc and HCl	S	195	8.4(0.05)	8.0(0.07)	7.3(0.36)	8.0(0.08)
		S	200	8.2(0.05)	8.2(0.12)	8.8(0.26)	8.2(0.07)

(Continued next page)

TABLE 6. (CONTINUED)

Compound	Species	Test Type[a]	Total Hardness (mg/L $CaCO_3$)	DO (mg/L)		pH	
				Start	End	Start	End
Solvent Yellow 33							
	Water flea	S	215	7.9(0.05)	7.7(0.21)	7.9(0.05)	7.9(0.05)
	Midge larva	S	215	7.9(0.09)	7.7(0.31)	7.8(0.05)	7.9(0.01)
	Mayfly larva	S	210	8.6(0.06)	8.0(0.36)	8.2(0.06)	7.8(0.06)
	Fathead minnow	S	210	7.9(0.05)	8.1(0.21)	8.2(0.06)	8.0(0.17)
	Rainbow trout	S	205	9.2(0.92)	7.3(0.35)	7.8(0.06)	7.7(0.23)
	Amphipod	S	192	9.2(0.23)	8.3(0.32)	8.2(0.06)	8.4(0.10)
	Bluegill	S	192	8.5(0.21)	4.0(0.09)	7.7(0.05)	7.6(0.05)
	Catfish	S	185	8.7(0.05)	6.7(0.84)	7.9(0.05)	7.5(0.18)
Solvent Green 3							
	Water flea	S	215	7.9(0.09)	7.7(0.21)	7.9(0.05)	7.8(0.05)
	Midge larva	S	215	7.9(0.05)	7.7(0.31)	7.8(0.09)	7.8(0.10)
	Mayfly larva	S	210	8.6(0.06)	7.2(0.75)	8.2(0.06)	7.7(0.05)
	Fathead minnow	S	210	7.8(0.14)	7.8(0.07)	8.2(0.06)	8.0(0.21)
	Rainbow trout	S	205	9.4(0.34)	7.1(0.31)	7.8(0.08)	7.6(0.15)
	Amphipod	S	192	9.4(0.06)	8.3(0.32)	8.2(0.12)	8.4(0.10)
	Bluegill	S	192	8.5(0.21)	4.0(0.09)	7.8(0.05)	7.6(0.09)
	Catfish	S	185	8.7(0.09)	6.9(0.68)	7.8(0.05)	7.4(0.09)

[a]S = static; SR = static renewal.

TABLE 7. ACUTE TOXICITY OF DEGDN TO EIGHT FRESHWATER AQUATIC ORGANISMS IN A STATIC TEST SYSTEM. TOXICITY VALUES BASED ON MEASURED AQUEOUS CONCENTRATIONS

Organism	Temperature (°C)	Test Endpoint	DEGDN (mg/L) LC50 (95% Fiducial limits)[a]
Water flea	22	48-h LC50	90.1 (74.0-109.6)
Midge larva	22	48-h LC50	159.8 (126.3-214.0)
Bluegill	22	96-h LC50	258.0 (223.7-297.2)
Channel catfish	22	96-h LC50	278.3 (239.0-322.5)
Rainbow trout	12	96-h LC50	284.1 (260.0-320.0)
Mayfly larva	22	48-h LC50	342.6 (299.4-380.0)
Amphipod	17	48-h LC50	355.3 (279.3-498.0)
Fathead minnow	22	96-h LC50	491.4 (447.2-564.4)

[a]Probit analysis.

TABLE 8. EFFECT OF DEGDN ON THE GROWTH OF Selenastrum capricornutum: CELLS/ML VS TEST DAY

Mean Measured Concentration (mg/L)	Test Day 0	1	2	3	4	5
Control	4,728[a]	21,213	89,605	268,424	530,281	1,211,239
58.4	4,943	15,868	30,727	92,805	140,063	243,751[b](-79.7)[c]
99.7	5,179	12,399	20,719	63,374	92,645	147,822[b](-87.6)
182.3	5,191	10,164	15,512	29,428	49,019	68,042[b](-94.4)
320.4	5,234	7,712	11,736	12,651	15,373	18,068[b](-98.5)
542.4	5,464	4,862	4,757	5,084	6,170	5,537[b](-99.5)

[a]Geometric mean of three replicate flasks; cells/mL.

[b]Significantly different from the controls (overall $p \leq 0.05$) at day 5. (Student's t test with Bonferroni's correction for simultaneous comparisons).

[c]Percent different from controls at day 5.

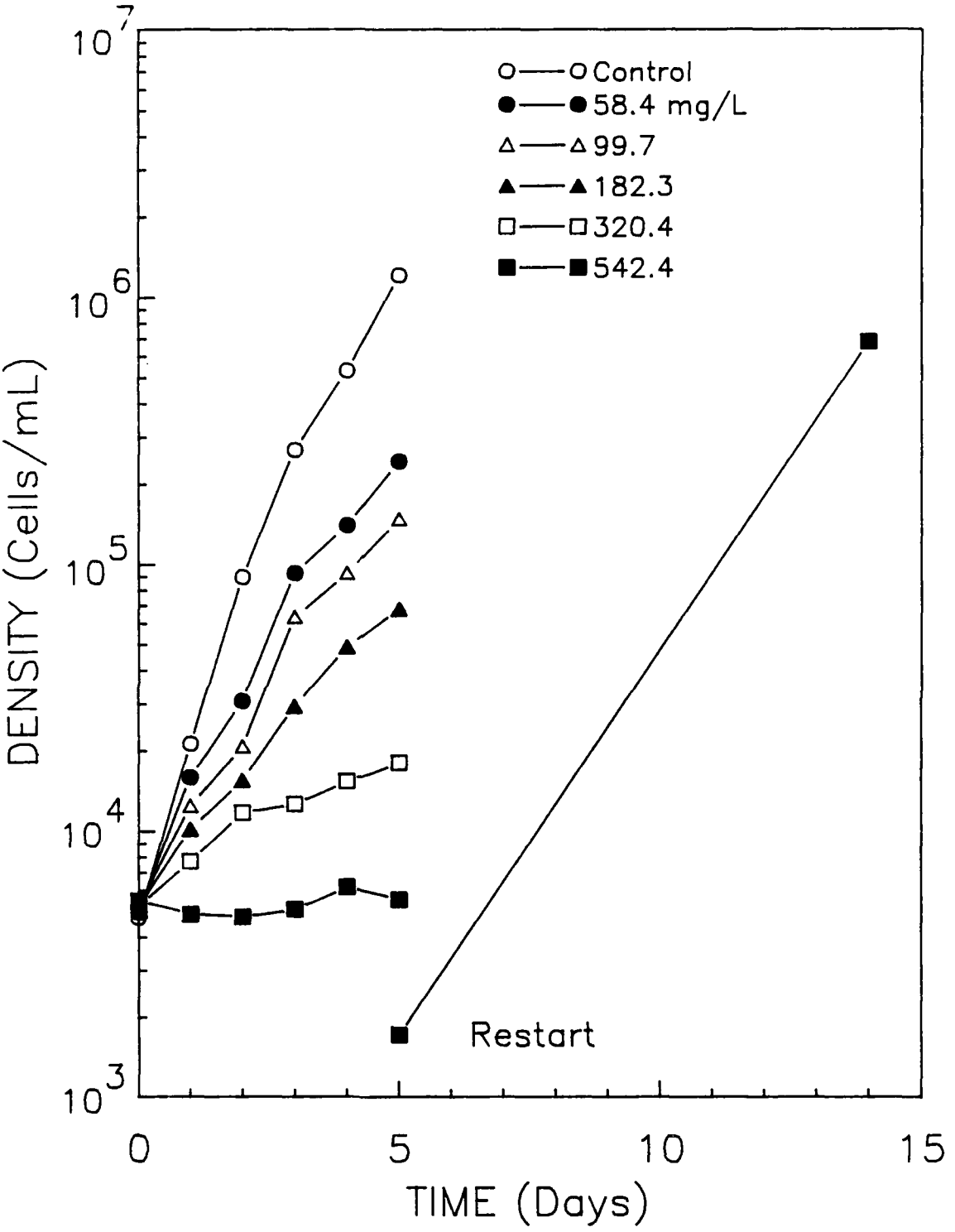

Figure 1. The effects of DEGDN on the growth of Selenastrum capricornutum as measured by density (Cells/mL). Restart was of rinsed cells in uncontaminated media.

TABLE 9. EFFECT OF DEGDN ON THE GROWTH OF Selenastrum capricornutum: RELATIVE CHLOROPHYLLa MEASUREMENTS

Mean Measured Concentration (mg/L)	Test Day 0	1	2	3	4	5
Control	0.62[a]	3.52	14.27	29.94	60.63	110.16
58.4	0.54	2.07	5.66	11.24	17.49	21.58[b](-80.1)[c]
99.7	0.62	1.50	3.52	6.40	9.57	12.47[b](-88.6)
182.3	0.58	1.78	1.67	3.18	4.87	4.13[b](-96.2)
320.4	0.57	1.04	1.23	1.82	1.20	0.99[b](-98.9)
542.4	0.48	0.93	0.72	0.73	0.50	0.41[b](-99.6)

[a]Geometric mean of three replicate flasks; µg/L chlorophyllα.

[b]Significantly different from the controls (overall $p \leq 0.05$) at day 5. (Student's t test with Bonferroni's correction for simultaneous comparisons).

[c]Percent different from controls at day 5.

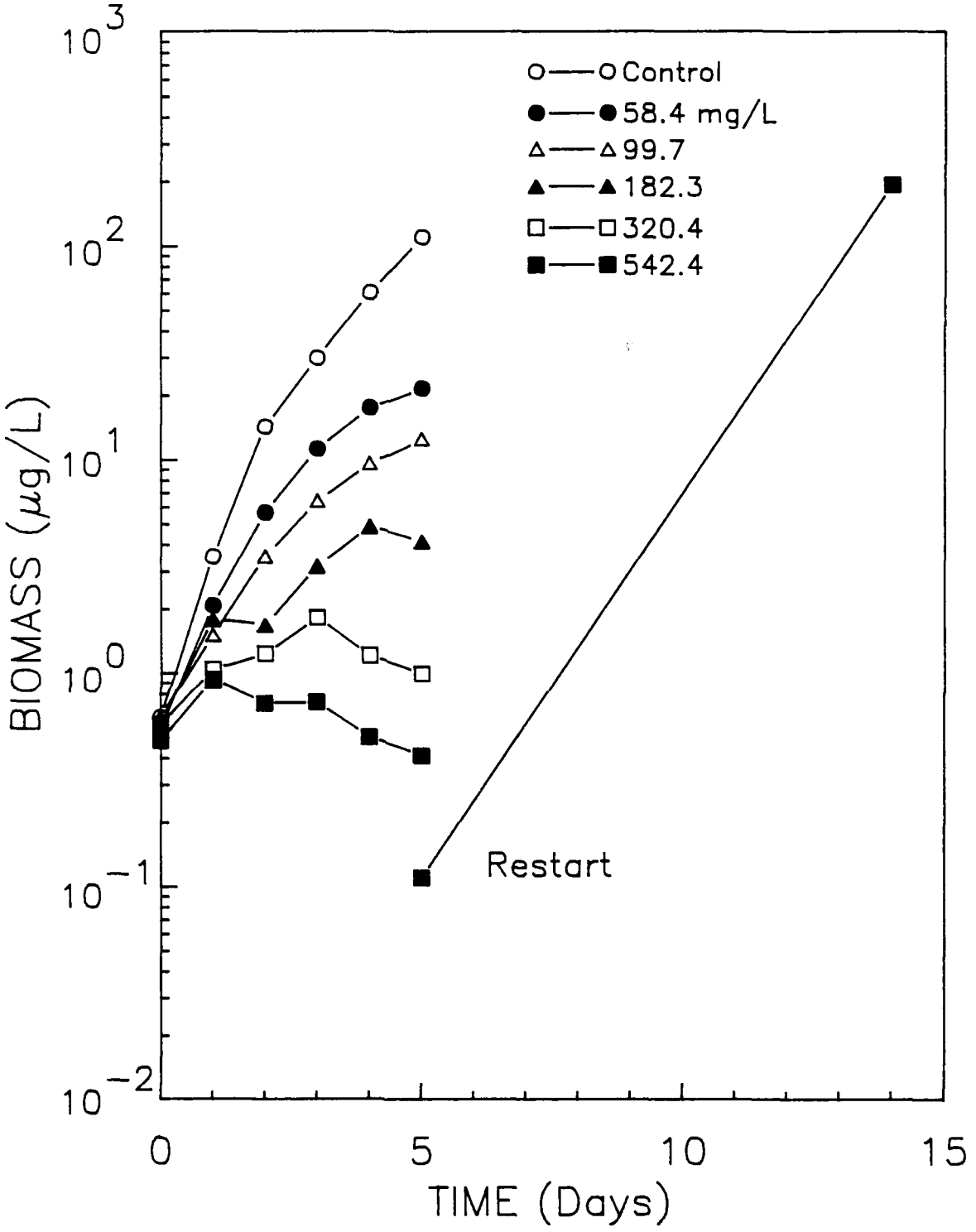

Figure 2. The effects of DEGDN on the growth of Selenastrum capricornutum as measured by biomass (µg/L Chlorophyll a). Restart was of rinsed cells in uncontaminated media.

nitroglycerin to be toxic to freshwater aquatic organisms in amounts ranging from 0.4 mg/L (5-day EC50 - growth) for Selenastrum capricornutum and 2.8 mg/L (96-h LC50) for Salmo gairdneri to 46 mg/L (48-h EC50) for Daphnia magna. For both compounds, the most sensitive species tested was Selenastrum capricornutum.

Algal species appear to be the most sensitive species for many nitrogen containing munitions products. Liu et al. (1984) found that the freshwater algae Microcystis aeruginosa and Selenastrum capricornutum were the most sensitive species to 2,4-DNT(dinitrotoluene) and a complex condensate wastewater mixture containing 31 different nitrogen containing compounds. The relative species sensitivities to nitrogen containing munitions seem to vary depending on the compound tested. Of the 31 compounds examined individually by Liu et al. (1984), Daphnia magna was more sensitive than Lepomis macrochirus in 14 cases. Bentley et al. (1978) found freshwater fish to be more sensitive than invertebrates to nitroglycerin. Liu et al. (1984) found the same trend of sensitivities with TNT (trinitrotoluene). Our toxicity studies with DEGDN indicate a more mixed sensitivity trend, with Daphnia magna being the most sensitive animal species but the rest of the invertebrates and fish showing varied sensitivities (Table 7). Fathead minnows proved to be the least sensitive species, while rainbow trout exhibited only average sensitivity (mean LC50 for all species = 282.5 mg/L; 96-h LC50 for rainbow trout = 284.1 mg/L).

Spanggord et al. (1985) found DEGDN to be very soluble in water (3900 mg/L). They indicated that aerobic and anaerobic biotransformation and photolysis appear to be the major processes which would determine the persistence of DEGDN in aqueous environments. In natural waters, they found biotransformation and photolytic half-lives of 40 and 27 days, respectively. The range of toxicities reported in this study indicate that DEGDN should not be a problem in most environments with respect to acute toxicity. Because of its high water solubility, it could pose a problem at production sites where the potential for spills exists.

4.3 Synthetic-HC Smoke Combustion Products Mixture

The concentrations of dissolved components in the 100% stock solutions varied between tests (Table 10). Values for the chlorinated organics were less for tests conducted later in the study (i.e., last four species in Table 10). An explanation for this anomaly is not apparent since, 1) all experimental and analytical conditions were the same throughout the study and 2) the metal concentrations did not change over the course of the study. The concentration of dissolved zinc remained relatively constant throughout the study (mean ± S.D. = 23.43 ± 2.3 mg/L). Zinc is by far the most abundant component in the stock mixture. Even after large dilutions of the stock mixture (i.e., 2 to 3% stock), the concentration of zinc still remained in the 1.0 to

TABLE 10. CONCENTRATIONS OF DISSOLVED COMPONENTS IN 100% STOCK SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURES FOR VARIOUS TOXICITY TESTS

Organism	Temperature (°C)	Test Type[a]	Component Concentration (mg/L)								
			CCl_4	C_2Cl_4	C_2Cl_6	C_6Cl_6	Zn	Pb	Cd	As	Al
Water flea	22	S & SR0	1.92	5.62	1.76	ND[b]	20.60	0.004	0.0240	0.0019	0.047[c]
		SR1	2.62	6.70	0.69	ND	24.90	0.005	0.0230	0.0016	0.047[c]
Midge larva	22	SR0	3.02	6.93	1.81	ND	19.20	0.003	0.020	0.0020	0.047[c]
		SR1	2.71	6.84	2.02	ND	25.00	0.005	0.025	0.0022	0.047[c]
Fathead minnow	22	SR0	2.09	6.87	1.26	ND	21.35	0.003	0.0225	0.0008	0.034
		SR1	2.33	7.39	1.50	ND	23.75	0.005	0.0236	0.0012	0.030
		SR2	2.16	6.98	0.79	ND	23.35	0.004	0.0215	0.0011	0.039
		SR3	2.28	7.17	1.45	ND	23.30	0.003	0.0227	0.0010	0.035
Mayfly larva	22	SR0	2.09	6.87	1.26	ND	21.35	0.003	0.0225	0.0008	0.034
		SR1	2.33	7.39	1.50	ND	23.75	0.005	0.0236	0.0012	0.030
Rainbow trout	12	SR0	2.17	7.76	1.02	ND	25.50	0.015	0.0300	0.0015	0.062
		SR1	1.97	7.26	1.03	ND	26.35	0.024	0.0247	0.0030	0.073
		SR2	2.12	7.40	0.91	ND	24.95	0.013	0.0258	0.0013	0.065
		SR3	2.17	7.58	1.17	ND	24.95	0.015	0.0240	0.0014	0.071
Channel catfish	22	S	0.82	2.84	0.33	ND	20.30	0.002	0.0244	0.0012	0.060
Bluegill	22	S	0.75	3.36	0.72	ND	23.60	0.003	0.0332	0.0015	0.047
Amphipod	17	S	0.75	3.36	0.72	ND	23.60	0.003	0.0332	0.0015	0.047
Alga	24	S	0.72	2.48	0.22	ND	28.00	0.024	0.0232	0.0006	0.069
		$\bar{X}$	1.95	6.16	1.12	ND	23.43	0.008	0.0249	0.0014	0.050
		SD	0.71	1.80	0.50		2.30	0.007	0.0035	0.0006	0.016

[a] S = static; SR = static renewal; Number = renewal number.
[b] ND = non-detectable (<0.01 mg/L C_6Cl_6).
[c] Estimated from earlier studies. New sample bottles contaminated with Al.

2.0 mg/L range (Appendix A). The relative consistency of the stock mixture over time can be seen in the identical toxic response of Daphnia magna tested at the beginning and end of the study (Table 13).

Actual measured component concentrations for all stocks and dilutions used during the study are presented in Appendix A. For some of the tests, analyses were conducted on the stock solutions only. The Appendix data shows good agreement between measured concentrations and concentrations predicted from dilutions of the measured stocks, especially for the more stable compounds. For example, measured zinc concentrations in the dilutions for the bluegill bioassay are similar to values predicted from the dilutions. There were considerable differences between measured and predicted values for the more unstable chlorinated organics.

The stability of the mixture varied according to the specific component examined and the elapsed time. The most extensive data concerning stability is from the rainbow trout test (Appendix A-3). These data show that after 24 h, most of the chlorinated organics had decreased in concentration by 64 to 82% in the 10% dilution. During that same period, the loss of metals ranged from 0 to 20%. An earlier study by Fisher et al. (1985) showed that the metals loss was due to precipitation from the dissolved phase. When a total sample was acidified after 48 h, it was found to contain the same amount of total metals as at the start. Further loss of the chlorinated organics was found after 96 h. For example, only 10% of the CCl_4 remained in solution at the end of the test. In contrast, there was little further loss of the metal components after 24 h.

The synthetic-HC smoke combustion products mixture was toxic to all organisms tested (Table 11). The most sensitive fish tested was the rainbow trout with a 96-h LC50 of 2.2% (95% fiducial limits = 1.9 - 2.7%) for a static test and 2.3% (2.0 - 2.7%) for a static renewal test. These values are based on the percentage of 100% stock solution in the dilutions. The most sensitive invertebrate appeared to be the water flea, with a 48-h LC50 of 9.3% (95% fiducial limits = 7.7 - 11.3%) for a static test and 9.6% (7.6 - 12.8%) for a static renewal test. The LC50 values for the two test types (static and static renewal) were not significantly different for either species.

The similarity between results from the two test types is indicative of toxicity due to metals. Miller et al. (1986) found the same LC50 for Ephemerella grandis exposed to a complex metals mixture whether the test was a 24-h static or a 96-h flow through. This is indicative of a rapid effect where most of the mortalities occur early in the exposure. This rapid early mortality was evident in the present study, regardless of the type of test. In both the daphnid and rainbow trout tests, the concentration of metals at the end of the tests were similar in the static and static renewal tests indicating a rapid decline in metals over the 24 h renewal period followed by stabilization

TABLE 11. ACUTE TOXICITY OF A SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE TO EIGHT FRESHWATER AQUATIC ORGANISMS. TOXICITY VALUES BASED ON DILUTIONS OF A 100% STOCK SOLUTION

Organism	Temperature (°C)	Type Test[a]	Syn-HC: % of Stock LC50 (95% Fiducial Limits)[b]
Rainbow trout	12	96 h; S	2.2 (1.9-2.7)
		96 h; SR	2.3 (2.0-2.7)
Water flea	22	48 h; S	9.3 (7.7-11.3)
		48 h; SR	9.6 (7.6-12.8)
Amphipod	17	48 h; S	<10[c]
Fathead minnow	22	96 h; SR	13.6 (10.1-16.7)
Channel catfish	22	96 h; S	42.3 (37.3-48.0)
Bluegill	22	96 h; S	45.9 (39.5-53.5)
Mayfly larva	22	48 h; SR	54.1 (46.8-62.6)
Midge larva	22	48 h; SR	89.0 (74.7-118.7)

[a]S = static; SR = static renewal.

[b]Probit analysis.

[c]Binomial method yielded an LC50 estimate of 6.32% wih 95% fiducial limits of 0 and 18%.

thereafter (Appendix A-1 and A-3). Also, the total metals in the test system did not change over the experimental period, as discussed earlier.

The amphipod also appeared to be quite sensitive to the mixture, although an LC50 value could not be calculated because the test was only designed as a range-finding bioassay. The test yielded 89% mortality at an 18% stock solution and 72% mortality at the greatest dilution tested (10% stock solution). These mortality data do not meet the criteria necessary to calculate an LC50 (Stephan 1978). LC50 values for the remaining fish species ranged from 13.6% for the fathead minnow to 45.9% for the bluegill. Values for the remaining invertebrates ranged from 54.1% for the mayfly larvae to 89% for the midge larvae.

Exposure to the synthetic-HC mixture for 5 days caused a dramatic reduction in Selenastrum capricornutum growth based on both density (Table 12; Figure 3) and biomass (Table 12; Figure 4). At the greatest dilution tested (5.6% stock), there was a reduction from control algal growth of 99.6% based on density (cells/mL) and 99.7% based on biomass (µg/L chlorophyll a). For all the dilutions tested (5.6, 10, 18, 32, 56 and 100% stock), the average reduction in density and biomass was 99.4% (S.D. = ± 0.34%) and 99.7% (S.D. = ± 0.31%), respectively. The measured component concentrations (mg/L) at the 5.6% treatment were: 0.02 - CCl_4; 0.06 - C_2Cl_4; 0.01 - C_2Cl_6; ≤ 0.005 - C_6Cl_6; 2.22 - Zn; 0.004 - Pb; 0.0029 - Cd; ≤0.0002 As; and 0.006 - Al. The lack of significant algal growth at the greatest dilution tested (5.6% stock) made it impossible to calculate a minimum algistatic concentration. Since the 5.6% stock treatment resulted in such a dramatic reduction in algal growth, it is safe to say that the algistatic concentration is well below this level. This indicates that Selenastrum capricornutum was the most sensitive species tested.

Algae cultures with measured densities ≤ 6500 cells/mL after 5 days exposure were restarted in fresh, uncontaminated media and allowed to recover for 9 days in an effort to determine an algicidal concentration. New cultures from the 5.6, 32, 56 and 100% treatments were restarted. At the end of the recovery phase, no culture resumed logarithmic growth (Figures 3 and 4). There was a slight, but statistically significant, increase in cell density in the culture from the 5.6% treatment (Table 12; Figure 3)(Student's t test, $p = 0.05$). In contrast, this culture showed no significant increase in biomass (Table 12; Figure 4). There was no measured growth in cultures from the other restarted treatments, based on either density or biomass. In fact, there was a decrease in biomass to non-detectable levels in all these cultures. These data indicate that the greatest dilution tested (5.6% stock) had an algicidal effect on Selenastrum capricornutum. Since there was an almost complete growth reduction of the alga at all dilutions tested, it was not possible to calculate a 5-day EC50.

TABLE 12. AVERAGE GROWTH OF Selenastrum capricornutum EXPOSED TO THE SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE-EXPRESSED AS DENSITY (CELLS/mL) AND BIOMASS (µG/L CHLOROPHYLL[a])

	Time							
	Day 0		Day 1		Day 2		Day 3	
Dilution	Den[a]	Bio[b]	Den	Bio	Den	Bio	Den	Bio
Control	5,196[c]	0.74	20,260	5.75	60,102	23.74	223,021	49.72
5.6	5,109	0.40	10,948	0.44	11,025	0.69	10,066	0.58
10	5,267	0.37	10,553	1.23	11,340	0.83	10,690	0.38
18	5,182	0.29	10,168	1.33	10,890	0.91	10,359	0.67
32	5,301	0.50	8,958	0.79	9,677	0.69	9,454	0.32
56	5,138	0.53	7,778	0.56	8,638	0.52	4,924	0.15
100	5,169	0.48	6,620	0.49	6,129	0.23	6,291	0.11

(Continued next page)

TABLE 12. (CONTINUED)

	Time							
	Day 4		Day 5		Restart		Day 14	
Dilution	Den	Bio	Den	Bio	Den	Bio	Den	Bio
Control	583,406	98.26	907032	84.49	-		-	
5.6	8,016	0.57	4,160[d]	0.29[d]	1,806	0.23	2,854[e]	0
10	9,699	0.74	8,465[d]	0.66[d]	-		-	
18	9,284	0.44	8,637[d]	0.43[d]	-		-	
32	6,194	0.22	5,944[d]	0.20[d]	3,511	0.18	3,545	0
56	4,932	0	3,984[d]	0[d]	3,586	0.07	3,967	0
100	4,383	0	5,735[d]	0[d]	3,026	0.02	2,365	0

[a]Den = density.

[b]Bio = biomass.

[c]Geometric mean of three replicate flasks.

[d]Significantly different from contols (overall $p \leq 0.05$) at day 5. (Student's t test with Bonferroni's correction for simultaneous comparisons).

[e]Significantly different from restart value (Student's t test, $p \leq 0.05$).

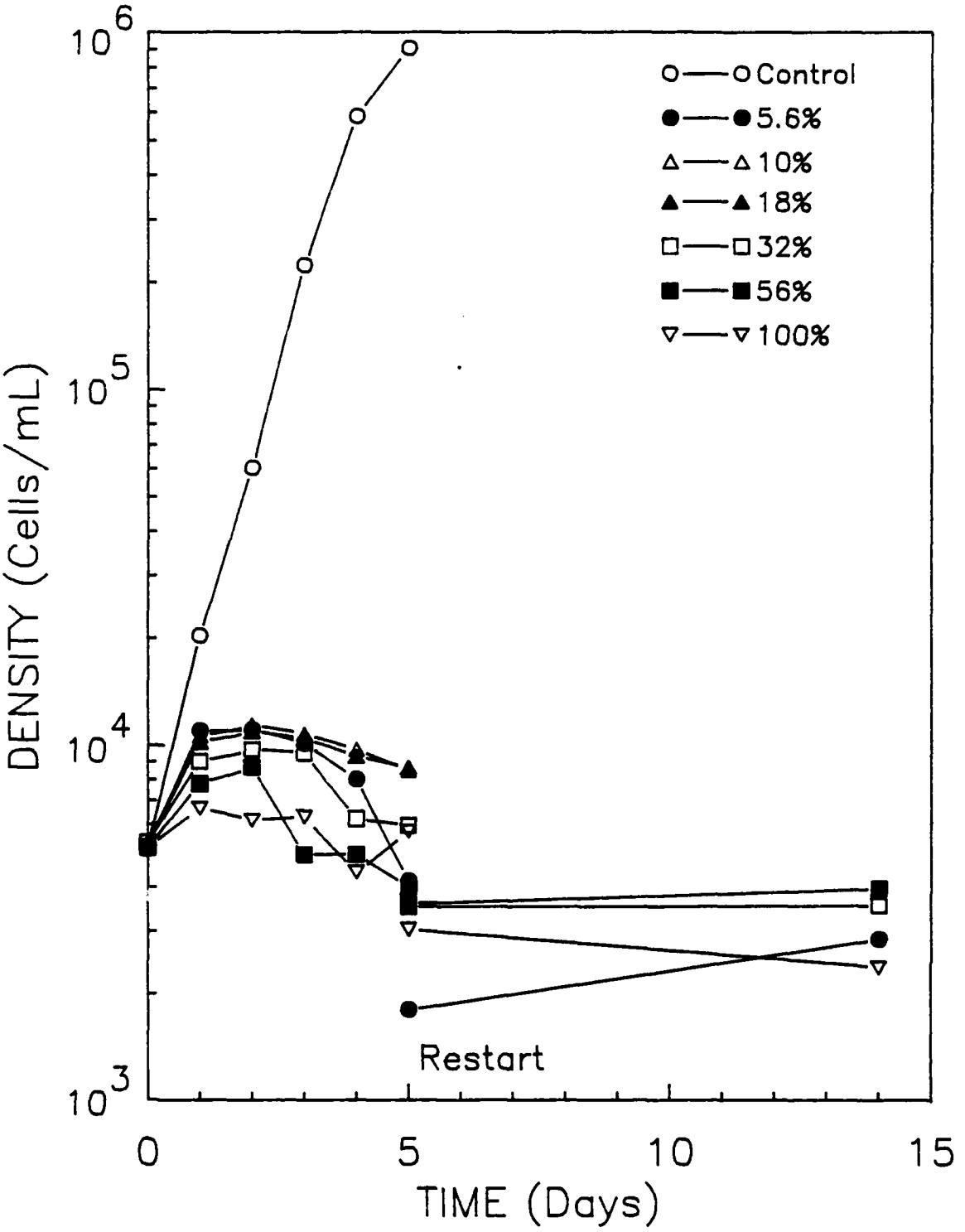

Figure 3. The effects of the synthetic-HC smoke combustion products mixture on the growth of Selenastrum capricornutum as measured by density (Cells/mL). Percentages are of stock mixture. Restart was of rinsed cells in uncontaminated media.

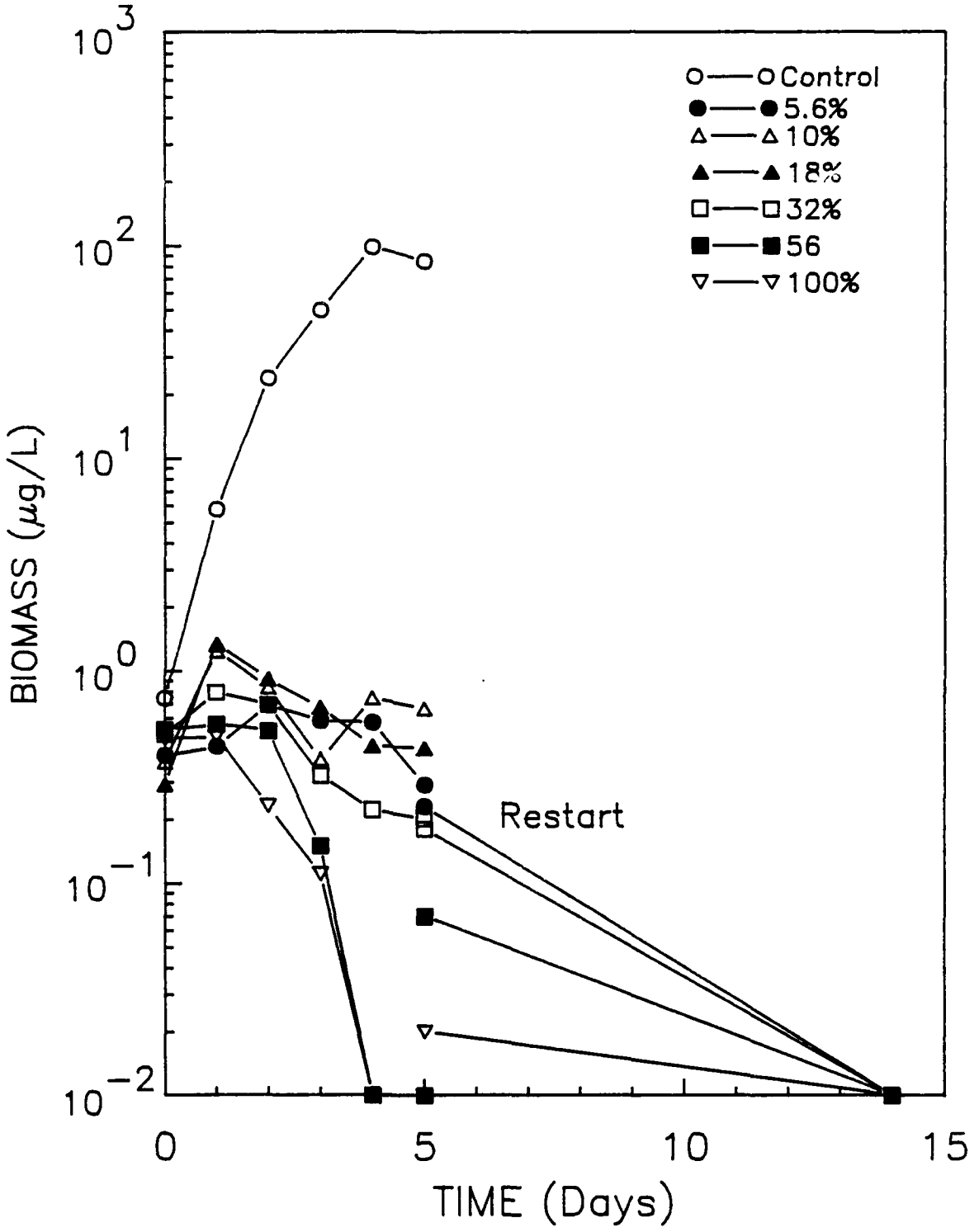

Figure 4. The effects of the synthetic-HC smoke combustion products mixture on the growth of Selenastrum capricornutum as measured by biomass (µg/L Chlorophyll a). Percentages are of stock mixture. Restart was of rinsed cells in uncontaminated media.

These toxicity data show that the dissolved components of this mixture are very toxic to a number of freshwater aquatic species, especially the alga Selenastrum capricornutum. A review of the literature indicates that this toxicity is not surprising considering the dominance of zinc in the mixture. Greene et al. (1975) found 14 day EC95 growth values of 40.4 and 68.0 µg Zn/L for this algal species, while Bartlett et al. (1974) showed growth inhibition of the same species at zinc levels of 30 ug/L. In the present study, the 5.6% stock solution treatment had a measured Zn concentration of 2.22 mg/L. According to the findings of Bartlett et al. (1974), even a dilution containing only 0.11% of the stock would have produced a measurable effect. This dilution would have been extremely dificult to obtain with any accuracy in the present study considering the test volumes used. The zinc concentration in the 5.6% treatment is well above the newest water quality criteria proposed by EPA (1985b) for the dilution water hardness used in the tests (criterion continuous concentration [CCC] = 147 µg/L; criterion maximum concentration [CMC] = 162 µg/L).

The data also indicate that zinc may be the component responsible for the toxicity of the mixture. Tests with individual and groups of components with daphnids seem to verify this (Table 13; Figure 5). Component concentrations for the stock solutions and the various dilutions used in these static tests are presented in Appendix A-5. Results of these tests indicate that the metals were the major toxic group, exhibiting toxicity equal to that of the total mixture. The tests also indicate that zinc was the major toxic individual component. Zinc tested alone yielded a 48-h LC50 of 12.8% (mean of two tests; S.D.s ranged from 9.3 to 17.2%). These percentages are based on dilutions of a Zn stock mixed using the same amount of Zn as in the total mix. This toxicity is similar to the average toxicity value of 9.0% (S.D.s ranged from 6.3 to 12.8%) from four tests with the total mix. When the chlorinated organics were tested as a group, they resuted in only minimal toxicity to the daphnids. Also, when the total mix was tested without zinc, the daphnids were much less sensitive with a 48-h LC50 of only 37.1%. The toxicity values from these component tests were also computed based on the measured zinc concentrations. The LC50 values computed in this manner were similar for the total mixture, Zn only and metals only tests, with average 48-h LC50 values of 1.83, 3.21 and 2.12 mg/L Zn, respectively (Table 14; Figure 6).

The tests with Daphnia magna indicate the possibility of some synergistic toxicity effect when the remaining metals are present with Zn. The total metals mixture had a 48-h LC50 of 8.1% (95% fiducial limits = 6.0 - 10.1%) while the zinc only mixture had LC50s of 13.8% (10.4 - 17.2%) and 11.7% (9.3 - 14.1%) in two separate tests. When these LC50 values were computed based on measured zinc concentrations (Table 14) it appeared that the daphnids were more sensitive to zinc in the presence of the other metals (e.g., LC50 for Zn lower when other metals present). Mixtures of metals have been shown to behave differently

TABLE 13. ACUTE TOXICITY OF SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE COMPONENTS TO *Daphnia magna*. BASED ON % OF STOCK SOLUTION

Test Organism	Date	Component	Duration	LC50 (95% Fiducial Limits)[a]	
				Static	S. Renewal
Daphnia	1/14/86	Total Mix	48h	9.3(7.7-11.3)	9.6(7.6-12.8)
	10/21/86			9.2(7.1-11.3)	
				8.0(6.3-9.7)	
	9/25/86	Zn and HCl		13.8(10.4-17.2)	
	10/2/86			11.7(9.3-14.1)	
	9/24/86	Metals Only		8.1(6.0-10.1)	
	9/25/86	Total minus Zn		37.1(31.0-43.2)	
	9/24/86	Organics Only		87.5(70.4-104.5)[b]	

[a]Probit analysis.

bTest did not meet ASTM guidelines for a valid bioassay since no dilution tested (including 100% stock solution) killed greater than 65% of the organisms. Probit program gave estimate. Binomial test gave statistically sound conservative 95% fiducial limits of 50% and + infinity for this data.

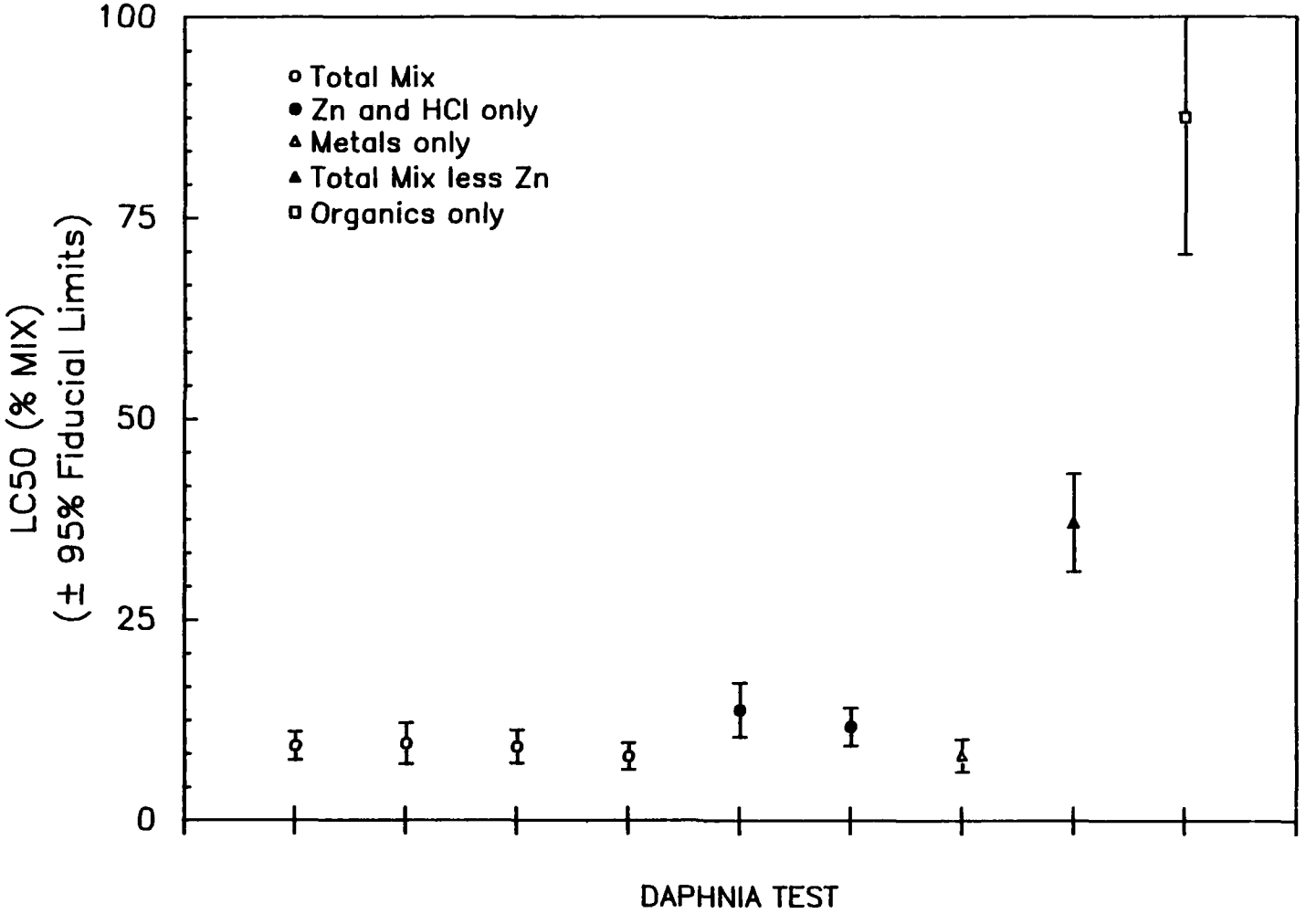

Figure 5. 48-h LC50s for Daphnia magna and various components of the synthetic-HC smoke combustion products mixture. LC50 values (probit analysis) are based on the percentages of a stock solution containing the specific test component or groups of components.

TABLE 14. ACUTE TOXICITY OF SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE COMPONENTS TO Daphnia magna. BASED ON MEASURED Zn CONCENTRATION (MG/L)

Test Organism	Date	Component	Duration	LC50 (95% Fiducial Limits)[a] Static	S. Renewal
Daphnia	1/14/86	Total Mix	48h	1.752(1.467-2.037)	1.857(1.402-2.312)
	10/21/86			1.968(1.576-2.360)	
				1.744(1.426-2.062)	
	9/25/86	Zn and HCl		3.575(2.715-4.435)	
	10/2/86			2.845(2.297-3.393)	
	9/24/86	Metals Only		2.116(1.591-2.641)	

[a]Probit analysis.

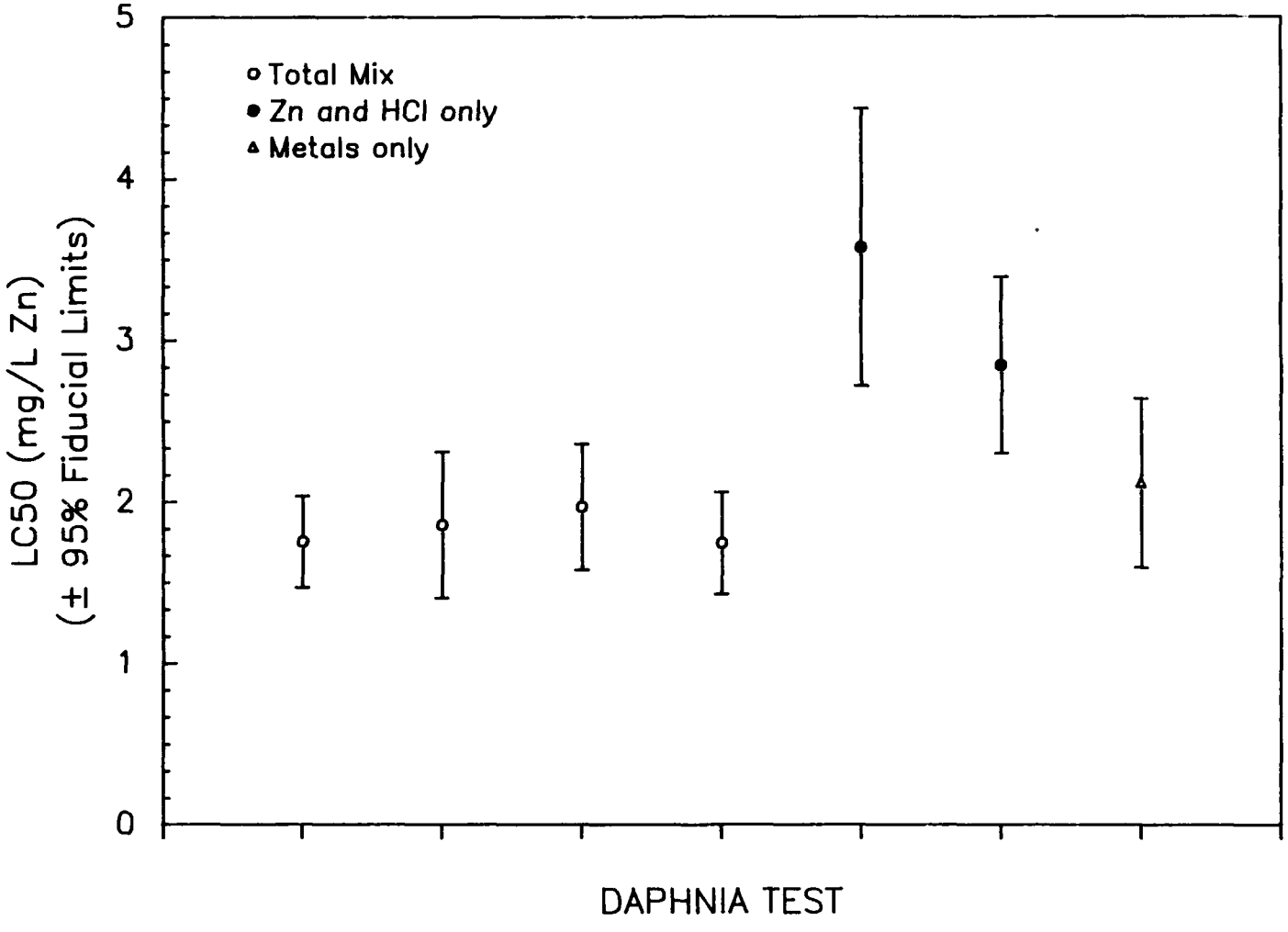

Figure 6. 48-h LC50s for Daphnia magna and various components of the synthetic-HC smoke combustion products mixture containing zinc. LC50 values (probit analysis) are based on measured Zn concentrations in the test dilutions.

toxicologically than their individual component metals (Wong and Beaver 1980; Wong et al. 1978; Eaton 1973; Sprague and Ramsey 1965). More information about the toxicity of the individual metals and their mixtures would be necessary to verify the interaction seen in the present study.

As noted above, Zn appears to be the major toxic component of this mixture, at least for the alga and daphnids. The 48-LC50 values for Daphnia magna based on measured Zn in the Zn only and total mix tests were 1.83 and 3.21 mg/L, respectively. These values are similar to zinc toxicity values reported in the literature for this species. The proposed national water quality criteria for zinc list a range of 48-h EC50s from 280 to 799 µg/L when the Zn was presented as $ZnCl_2$, as in the present study (USEPA 1986b). These values were based on immobilization rather than death and are therefore not directly comparable. Since the toxicity of zinc is quite variable, depending on a number of chemical factors (i.e., calcium, magnesium, hardness, pH and ionic strength), toxicity values will vary considerably between studies (USEPA 1986b).

Other than zinc, the concentrations of the remaining metals in the mixture appear to be below levels which would be considered acutely toxic. The national water quality critera (USEPA 1985a) for arsenic are 0.19 and 0.36 mg/L for CCC and CMC, respectively, while the average concentration in the 100% stock solutions was only 0.0014 mg/L. The lowest species mean acute value (SMAV) given for lead in the national water quality criteria is 0.15 mg/L for Gammarus pseudolimnaeus (USEPA 1985b). Other SMAVs listed in the same document were 0.45, 2.45, 25.44 and 52.31 mg/L for daphnids, rainbow trout,fathead minnow and bluegill, respectively. In contrast, the concentration of lead in the 100% stock solutions averaged only 0.008 mg/L. SMAVs for cadmium reported in the national water quality criteria range from a low of 0.0036 mg/L for rainbow trout to 6.96 mg/L for bluegill (USEPA 1986c). Daphnia magna was the second most sensitive species reported, with a SMAV of 0.013 mg/L. Since the concentration of cadmium in the stock solutions for all tests averaged 0.025 mg/L, there is a possibility for acute cadmium toxicity from the stock solutions, especially to daphnids and rainbow trout. If cadmium concentrations are examined at the LC50 dilutions though, it can be seen that they are below the reported SMAV (i.e., 0.0008 mg/L at the LC50 dilution for rainbow trout and 0.004 mg/L for daphnids). Burrows (1977) reports toxic levels of aluminum ranging from 0.32 mg/L for Daphnia magna to 5 mg/L for Salmo gairdneri. Aluminum concentrations in the stock solutions in the present study averaged only 0.05 mg/L.

Similarly, the chlorinated organic compounds also seem to be present in amounts that should not be acutely toxic, especially at dilutions of the stock mixtures which caused toxicity. When these organics were tested as a group, they exhibited only minimal toxicity to Daphnia magna (Table 13; Figure 5). Carbon tetrachloride does not appear to be acutely toxic to daphnids at

concentrations below 27.3 mg/L (USEPA 1978). In fact, a chronic no-effects level for Pimephales promelas was reported to be 3.4 mg/L in this same document. In the 100% stock solutions, the average concentration of carbon tetrachloride was only 1.95 mg/L (S.D. = ± 0.71 mg/L) Shubat et al. (1982) found a 96-h LC50 of 4.99 mg/L for Salmo gairdneri using tetrachloroethylene. Both LeBlanc (1980) and Richter et al. (1983) found a 48-h LC50 of 18.0 mg/L for this compound with Daphnia magna, while Walbridge et al. (1983) found a 96-h LC50 of 13.4 mg/L for the fathead minnow. Although the average concentration of tetrachloroethylene in the 100% stocks in the present study was 6.16 mg/L (S.D. = ± 1.80 mg/L), the measured concentrations were well below acutely toxic levels at dilutions near the LC50. For example, the 48-h LC50 for Daphnia magna was 9.0%. The measured concentration of this organic compound in the 10% dilution of this test was 0.45 mg/L, well below levels found toxic by Leblanc (1980) and Richter et al. (1983).

The national water quality criteria for hexachloroethane indicates a most sensitive species SMAV of 0.98 mg/L for both rainbow trout and bluegill (USEPA 1980b). At dilutions near the LC50 values for these species though, hexachloroethane concentrations were found to be only 0.023 mg/L for rainbow trout and 0.24 mg/L for bluegills. In contrast to the other chlorinated organics, hexachlorobenzene was never detected in the stock mixtures, even when the detection limit was as low as 0.002 mg/L. Calamari et al. (1983) found only a 12% growth inhibition of Selenastrum capricornutum at 0.03 mg/L hexachlorobenzene, and no toxicity to Daphnia magna or Salmo gairdneri at the same concentration. Laska et al. (1978) found no mortality in crayfish (Procambarus clarki) or largemouth bass (Micropterus salmoides) after 10 days of exposure to 0.027 mg/L and 0.026 mg/L hexachlorobenzene, respectively.

Species sensitivities to the synthetic-HC mixture were also consistent with zinc toxicity. The alga was the most sensitive species, followed by the rainbow trout and the daphnids. This is the same general sensitivity trend reported in the national water quality criteria for zinc (USEPA 1986b). For tests conducted at similar water hardnesses, this document reports a 7-day growth inhibition value for Selenastrum capricornutum of 30 µg/L Zn, a 96-h LC50 for swim-up stage rainbow trout of 93 - 136 µg/L and a 48-h EC50 for Daphnia magna of 334 µg/L.

The results of the present study indicate that, at the component concentrations tested, the water soluble fraction of the synthetic-HC smoke combustion products would be a serious threat to freshwater aquatic species. Every indication is that zinc is the major toxic component of this mixture. It must be stressed that these component concentrations are artificially derived levels which were used in an effort to determine LC50 values for the various species. At present, there is no data concerning the actual amounts of these components which could end up in the aquatic environment after use of the grenades and smoke

pots or after disposal of the stockpiled munitions. These type of data are essential for determining potential hazards to aquatic organisms.

4.4 Solvent Yellow 33 and Solvent Green 3

Fisher et al. (1985) found the solubility of solvent yellow 33 to range from 0.09 mg/L at 12°C to 0.17 mg/L at 22°C, whether it was alone or part of the solvent green 3 mixture. The green component of the solvent green 3 was never detected in water samples, even when detection limits were reduced to 0.002 mg/L using a C_{18} solid phase extraction cartridge for concentration. During the toxicity tests, the average concentrations of solvent yellow 33 at its solubility limits were 0.20 mg/L (S.D. = ± 0.013 mg/L), 0.16 mg/L (± 0.031), 0.12 mg/L (± 0.009) and 0.09 mg/L (± 0.009) in tests conducted at 24, 22, 17 and 12°C, respectively (Table 15). For the yellow 33 component of the solvent green 3, concentrations at the same test temperatures averaged 0.20 mg/L (S.D. = ± 0.030 mg/L), 0.16 mg/L (± 0.031), 0.10 mg/L (± 0.001) and 0.08 mg/L (± 0.004), respectively. The green component of this dye mixture was never detected during the toxicity tests (detection limit = 0.002 mg/L). During the rainbow trout test, the 50% dilution of the solvent green 3 solubility stock was found to contain 0.055 mg/L (S.D. = ± 0.0005 mg/L) of the yellow 33 component and non-detectable amounts of the green component.

All of the vertebrate and invertebrate species were tested at the solubility limit of each dye. Only during the rainbow trout test was there significant mortality compared to the controls (Table 15). The solvent green 3 killed 50% of the trout exposed at the solubility limit in two separate tests. There were no mortalities at a 50% dilution of this mix. Due to the lack of both sufficient partial kills and a complete kill, it was impossible to calculate an LC50. The solvent yellow 33 did not cause any mortality to the rainbow trout. Concentrations above the solubility limit were not tested (i.e., no carrier solvents).

There was a significant effect when Selenastrum capricornutum was exposed to either dye. Following 5 days exposure to solvent yellow 33 at its solubility limit, cell density was reduced 68% from control values, while biomass was reduced 75% (Table 16; Figures 7 and 8). After the same exposure period, solvent green 3 caused a 98% reduction in cell density and a 99% reduction in biomass compared to controls. For both dyes, cell density values after 5 days were significantly greater than values at the start, indicating that neither an algistatic or algicidal concentration had been tested (Table 16). Since the algae were tested only at the solubility limit of the dyes, no EC50 could be calculated. Because of its dramatic effect, it is obvious that the solvent green 3 EC50 for the alga is well below its solubility limit. Thus, Selenastrum capricornutum is the most sensitive species tested with the dyes. In fact, this alga

TABLE 15. ACUTE TOXICITY OF SOLVENT YELLOW 33 (SY33) AND SOLVENT GREEN 3 (SG3) TO EIGHT FRESHWATER AQUATIC ORGANISMS. TESTS WERE CONDUCTED BY STATIC PROCEDURES

Organism	Temperature (°C)	Test	Measured dye concentrations (mg/L)			% Mortality	
			SY33	SG3[a] Yellow	SG3[a] Green	SY33	SG3
Water flea	22	48-h S.L.[b]	0.17	0.18	<0.08	0	0
Midge larva	22	48-h S.L.	0.17	0.18	<0.08	0	0
Bluegill	22	96-h S.L.	0.12	0.12	<0.08	0	0
Channel catfish	22	96-h S.L.	0.11	0.11	<0.08	0	0
Rainbow trout	12	96-h S.L.	0.089	0.076	<0.002	0	50
		96-h 50% S.L.	--	0.055	<0.002	-	0
Mayfly larva	22	48-h S.L.	0.18	0.17	<0.08	0	0
Amphipod	17	48-h S.L.	0.12	0.10	<0.08	0	0
Fathead minnow	22	96-h S.L.	0.18	0.17	<0.08	0	0

[a]Solvent green 3 is composed of a yellow (solvent yellow 33) component and a green component.

[b]S.L. = solubility limit.

TABLE 16. AVERAGE GROWTH OF Selenastrum capricornutum EXPOSED TO SOLVENT YELLOW 33 AND SOLVENT GREEN 3 EXPRESSED AS DENSITY (CELLS/ML) AND BIOMASS (µG/L CHLOROPHYLL a)

	Time							
	Day 0		Day 1		Day 2		Day 3	
Treatment	Den[a]	Bio[b]	Den	Bio	Den	Bio	Den	Bio
Yellow 33								
Control	4,728[c]	.62	21,213	3.52	89,605	14.27	268,424	29.94
Solubility Limit	4,302	.99	22,053	4.71	84,150	9.82	150,319	15.55
Green 3								
Control	5,196	.74	20,260	5.75	60,102	23.74	223,021	49.72
Solubility Limit	5,239	.50	11,982	1.57	11,106	1.28	12,480	.51

(Continued next page)

TABLE 16. (CONTINUED)

Treatment	Time			
	Day 4		Day 5	
	Den	Bio	Den	Bio
Yellow 33				
Control	530,281	60.63	1,211,239	110.16
Solubility Limit	284,777	18.28	383,774	25.26
Green 3				
Control	583,406	98.26	907,032	84.49
Solubility Limit	14,889	0.84	17,208	0.51

[a]Den = Density.

[b]Bio = Biomass.

[c]Geometric mean of three replicate flasks.

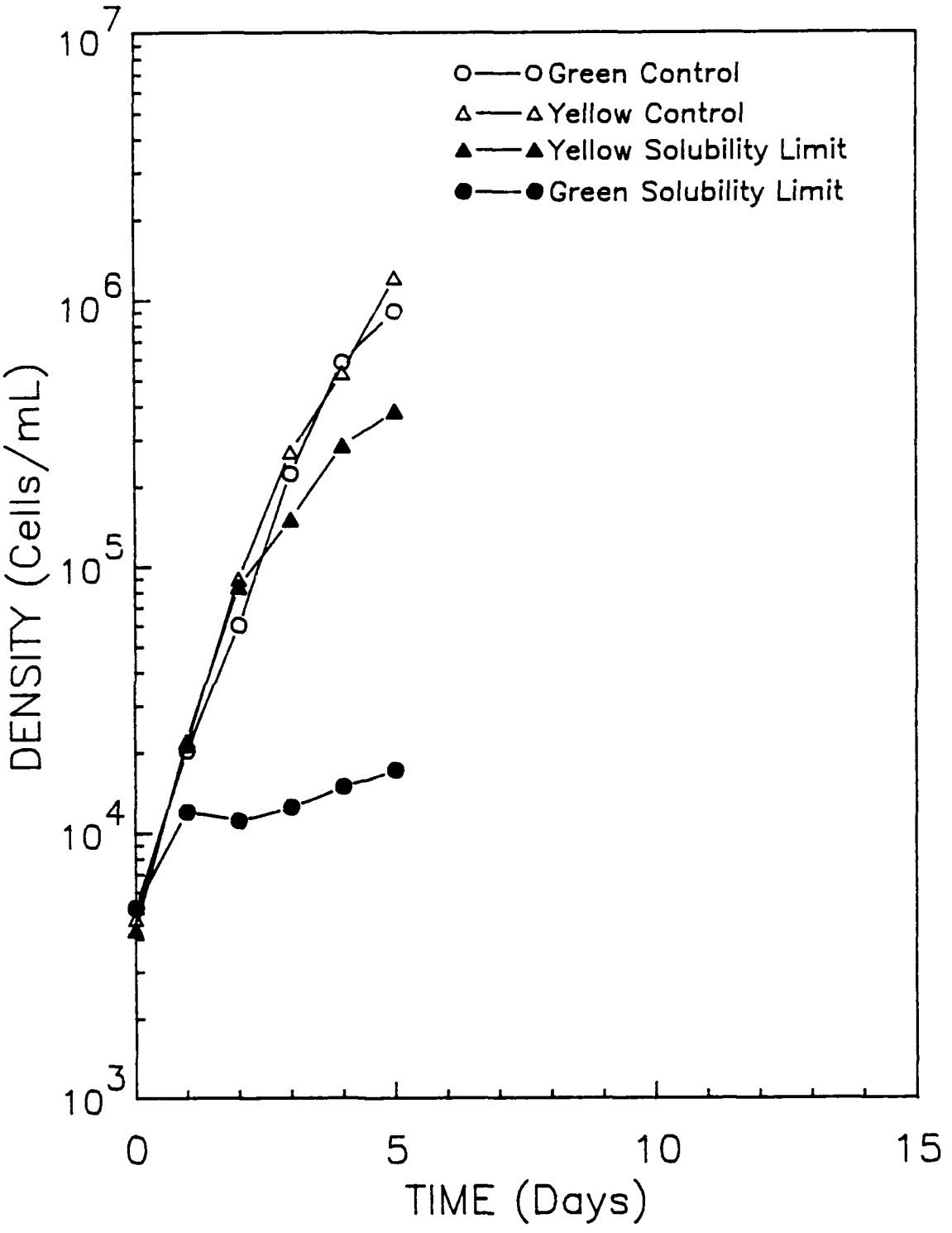

Figure 7. The effects of solvent yellow 33 and solvent green 3 at their solubility limits on Selenastrum capricornutum growth as measured by density (Cells/mL). No cultures were restarted in uncontaminated media since all had ≥ 6500 cells/mL after 5 days.

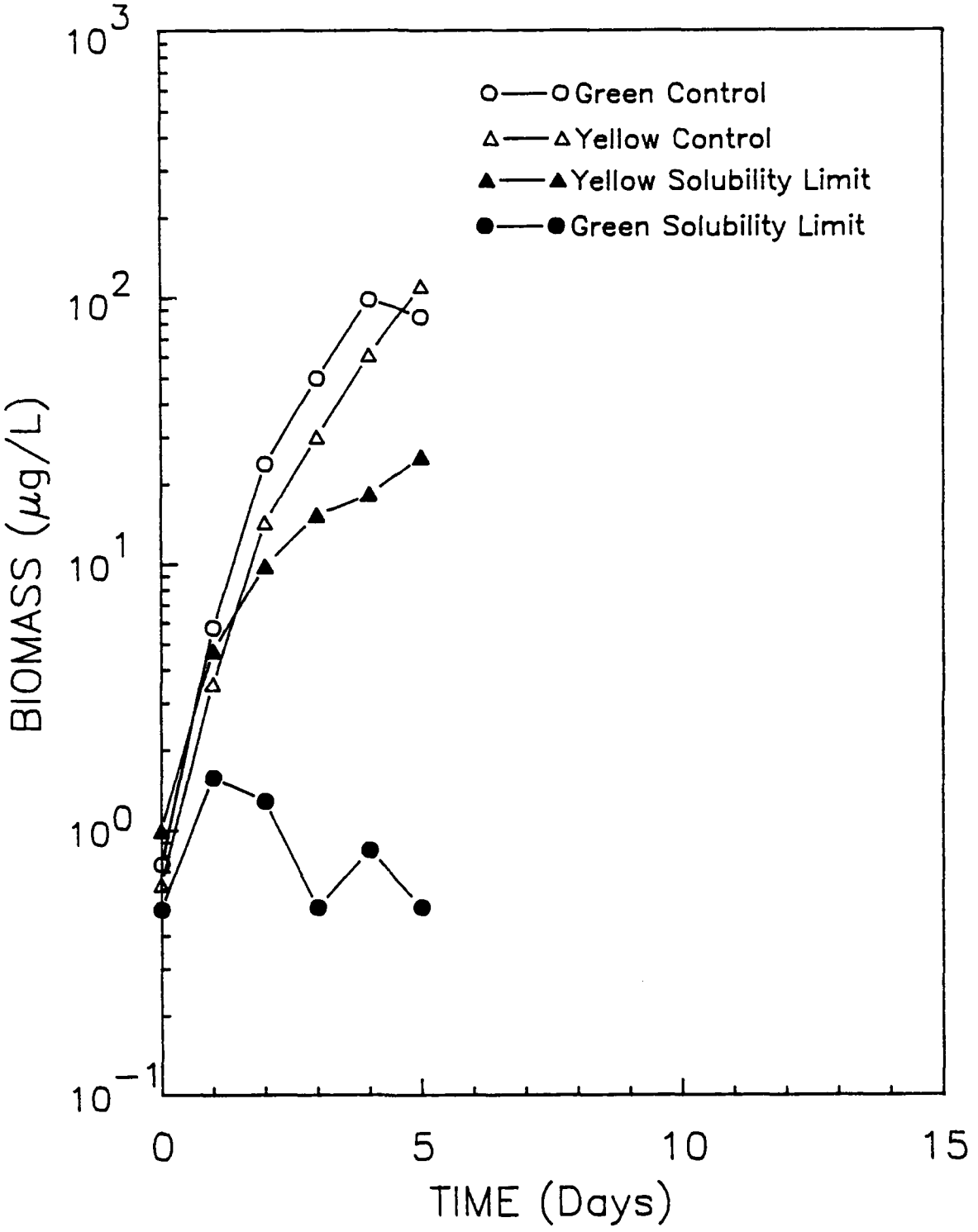

Figure 8. The effects of solvent yellow 33 and solvent green 3 at their solubility limits on Selenastrum capricornutum growth as measured by biomass (µg/L Chlorophyll a). No cultures were restarted in uncontaminated media since all had ≥ 6500 cells/mL after 5 days.

was the only freshwater species tested which was affected by the solvent yellow 33.

There appears to be a potential environmental problem with these dyes, especially solvent green 3. Although there was no acute toxicity caused by either dye to seven of the nine species tested, there was an effect on the rainbow trout and the alga. The effect of solvent green 3 on Selenastrum capricornutum was especially dramatic considering the aqueous concentration at the solubility limit. Whenever a compound causes mortalities in the low ppm (0.2 mg/L yellow 33 component) and ppb ($\leq$ 0.002 mg/L) range, even to only a few species, concern must be expressed. Usage, production and disposal patterns should be examined to determine both the existing levels of environmental contamination and the possibility of environmental release of these dyes.

Technical formulations of both dyes contained one major impurity which could possibly be the cause of the toxicity seen. Henderson et al. (1984) examined the dyes and found that this impurity made up 2.2%, by weight, of the solvent yellow 33 and 0.7% of the solvent green 3. They identified it as a condensation of one molecule of quinaldine with two molecules of phthalic anhydride. If this impurity was the cause of the dramatic alga effect seen with the solvent green 3, a similar effect should have been seen with the solvent yellow 33 since the impurity is present in even greater amounts. Also, this impurity did not seem to show a differentially greater solubility than the actual dye components in our HPLC samples. Therefore, it appears that the possible toxic effects of this impurity are minimal.

SECTION 5

CONCLUSIONS

The toxicity of DEGDN was relatively low to the nine freshwater species tested, especially when compared to other commonly used nitrate ester explosives such as nitroglycerin and ethyleneglycol dinitrate. Toxicity values ranged from a 5-day EC50 (standing crop) of 39.1 mg/L for the green alga Selenastrum capricornutum, to a 96-h LC50 of 491.4 mg/L for the fathead minnow (Pimephales promelas). The most sensitive invertebrate tested was Daphnia magna which was more sensitive than the most sensitive fish tested, Lepomis macrochirus. Due to its high water solubility, DEGDN could cause environmental problems following significant introductions to receiving streams at production facilities.

The dissolved components of the synthetic-HC smoke combustion products mixture were found to be quite toxic to a number of freshwater species, especially Selenastrum capricornutum, Salmo gairdneri and Daphnia magna. A test solution containing only 5.6% of a stock mixture of these components caused both an algistatic and algicidal effect on the alga. LC50 values for the trout and the water flea were 2.2% and 9.3% of the stock solution, respectively. Additional tests with the water flea indicate that zinc was the major toxic component of the mixture. Information concerning environmental concentrations of the various components after use or disposal of the munitions is necessary in order to assess possible hazards to aquatic life.

Solvent yellow 33 and solvent green 3 were not toxic to seven of nine freshwater species when tested at their solubility limits. A solubility limit solution of solvent green 3 killed 50% of the rainbow trout exposed but was non-toxic when diluted by 50%. Solvent yellow 33 was non-toxic to the trout at its solubility limit. Both dyes caused a reduction in alga growth at solubility limits, with solvent green 3 being most detrimental, causing a 98 - 99% reduction after 5 days of exposure. These dyes could cause a problem if released to the environment since an effect was noted at their low solubility concentrations (i.e., 0.2 mg/L solvent yellow 33 and < 0.002 mg/L green component of solvent green 3).

SECTION 6

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APPENDIX A

DISSOLVED CONCENTRATIONS OF THE COMPONENTS OF THE SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE IN DILUTIONS USED DURING TOXICITY TESTS

- unless otherwise stated, ND means non detectable, as follows:

Compound	Detection limit (mg/L)
Chlorinated organics	0.01*
Zn	0.00008
Pb	0.0002
Cd	0.0002
As	0.0002
Al	0.002

*The purchase of a new computer/intergrator system allowed better sensitivity and a detection limit of 0.005 mg/L in some instances

A-1. CONCENTRATIONS OF DISSOLVED COMPONENTS OF SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE DURING *Daphnia magna* STATIC AND STATIC RENEWAL TESTS

Sample	Component Concentration (mg/L)								
	CCl_4	C_2Cl_4	C_2Cl_6	C_6Cl_6	Zn	Pb	Cd	As	Al
Start of Static and Static Renewal									
100% Stock	1.922	5.617	1.763	ND	20.600	0.004	0.0240	0.0019	[a]
1.8%	0.012	0.043	0.013	ND	0.647	ND	0.0015	ND	
3.2%	0.028	0.111	0.028	ND	0.780	ND	0.0014	0.0003	
5.6%	0.079	0.248	0.067	ND	1.090	ND	0.0025	0.0005	
10%	0.158	0.451	0.139	ND	1.860	ND	0.0040	0.0003	
18%	0.277	0.658	0.263	ND	3.120	ND	0.0058	0.0005	
End 1st 24-h Static Renewal									
1.8%	ND	0.019	0.010	ND	0.243	ND	0.0004	0.0004	
3.2%	0.015	0.072	0.023	ND	0.374	ND	0.0007	ND	
5.6%	0.018	0.098	0.034	ND	0.753	ND	0.0017	0.0003	
10%	0.045	0.228	0.088	ND	1.330	ND	0.0030	0.0005	
18%	0.108	0.407	0.170	ND	2.070	ND	0.0050	0.0003	
Start 2nd 24-h Static Renewal									
100% Stock	2.623	6.704	0.694	ND	24.900	0.005	0.0230	0.0016	
1.8%	0.014	0.057	ND	ND	0.333	ND	0.0007	0.0006	
3.2%	0.031	0.123	0.012	ND	0.551	ND	0.0013	0.0006	
5.6%	0.087	0.272	0.020	ND	1.170	ND	0.0022	0.0003	
10%	0.170	0.477	0.040	ND	1.900	ND	0.0036	0.0004	
18%	0.337	0.745	0.100	ND	3.620	ND	0.0062	0.0007	
End 48-h Static Renewal									
1.8%	0.010	0.036	ND	ND	0.276	ND	0.0004	ND	
3.2%	0.014	0.063	ND	ND	0.482	ND	0.0009	ND	
5.6%	0.042	0.176	0.016	ND	0.978	ND	0.0013	0.0004	
10%	0.088	0.293	0.027	ND	1.480	ND	0.0017	0.0007	
18%	0.152	0.476	0.053	ND	2.310	ND	0.0046	0.0007	

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A-1. (CONTINUED)

Sample	Component Concentration (mg/L)								
	CCl_4	C_2Cl_4	C_2Cl_6	C_6Cl_6	Zn	Pb	Cd	As	Al
End 48-h Static									
1.8%	0.010	0.034	0.011	ND	0.234	ND	0.0004	0.0004	[a]
3.2%	0.014	0.063	0.019	ND	0.365	ND	0.0008	0.0002	
5.6%	0.029	0.140	0.041	ND	0.723	ND	0.0013	ND	
10%	0.030	0.158	0.061	ND	0.945	ND	0.0024	0.0005	
18%	0.099	0.370	0.143	ND	1.820	ND	0.0049	0.0006	

[a]No aluminum samples due to aluminum contamination of new sample bottles. 0.047 mg/L should represent a realistic estimate of the concentration of Al in 100% mixture.

A-2. CONCENTRATIONS OF DISSOLVED COMPONENTS OF SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE DURING Paratanytarsus parthenogeneticus STATIC RENEWAL TEST

Sample	Component Concentration (mg/L)								
	CCl_4	C_2Cl_4	C_2Cl_6	C_6Cl_6	Zn	Pb	Cd	As	Al
100% Stock-Start of test-Rep 1	2.915	6.903	1.765	ND[a]	19.200	0.003	0.0220	0.0020	b
100% Stock-Start of test-Rep 2	3.118	6.958	1.845	ND	19.200	0.003	0.0220	0.0020	
100% Stock-Start of 2nd 24-h	2.705	6.841	2.018	ND	25.0	0.005	0.025	0.0022	

[a]ND = non-detectable: <0.01 mg/L C_6Cl_6.

[b]No aluminum sample due to aluminum contamination of new sample bottles. 0.047 mg/L should represents a realistic estimate of the concentration of Al in 100% mixture.

A-3. CONCENTRATIONS OF DISSOLVED COMPONENTS OF SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE DURING Salmo gairdneri STATIC AND STATIC RENEWAL TESTS

Sample	Component Concentration (mg/L)								
	CCl_4	C_2Cl_4	C_2Cl_6	C_6Cl_6	Zn	Pb	Cd	As	Al
Start of Static and Static Renewal									
100% Stock	2.165	7.765	1.020	ND[a]	25.50	0.015	0.0300	0.0015	0.062
1.0%	0.012	0.047	0.007	ND	0.29	ND[a]	0.0005	ND[a]	0.002
1.8%	0.037	0.114	0.014	ND	0.50	ND	0.0006	0.0004	0.003
3.2%	0.039	0.122	0.016	ND	0.85	ND	0.0009	0.0004	0.005
5.6%	0.083	0.222	0.033	ND	1.76	ND	0.0016	0.0003	0.006
10.0%	0.154	0.536	0.076	ND	2.49	ND	0.0030	0.0005	0.009
End 1st 24-h Static Renewal									
1.0%	0.006	0.019	ND[a]	ND	0.28	ND	0.0005	ND	0.003
1.8%	0.010	0.032	ND	ND	0.45	ND	0.0006	ND	0.003
3.2%	0.014	0.056	0.005	ND	0.75	ND	0.0008	ND	0.004
5.6%	0.026	0.083	0.009	ND	1.54	ND	0.0017	0.0005	0.006
10%	0.055	0.140	0.014	ND	2.27	ND	0.0026	0.0004	0.009
Start of 2nd 24-h Static Renewal									
100% Stock	1.970	7.256	1.028	ND	26.35	0.024	0.0247	0.0030	0.073
1.0%	0.012	0.046	0.005	ND	0.29	ND	0.0004	ND	0.002
1.8%	0.028	0.087	0.010	ND	0.51	ND	0.0005	0.0004	0.003
3.2%	0.058	0.157	0.019	ND	0.77	ND	0.0007	0.0003	0.005
5.6%	0.104	0.337	0.044	ND	1.47	ND	0.0014	0.0005	0.006
10.0	0.153	0.513	0.068	ND	2.27	ND	0.0024	0.0008	0.009
End 48-h Static Renewal[b]									
1.0%	ND[a]	0.017	ND	ND	0.30	ND	0.0003	ND	0.002
1.8%	0.009	0.031	ND	ND	0.58	ND	0.0004	0.0004	0.003
3.2%	0.019	0.068	0.005	ND	0.82	ND	0.0008	ND	0.005

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A-3. (CONTINUED)

Sample	Component Concentration (mg/L)								
	CCl_4	C_2Cl_4	C_2Cl_6	C_6Cl_6	Zn	Pb	Cd	As	Al
Start 3rd 24-h Static Renewal									
100% Stock	2.120	7.400	0.911	ND	24.95	0.013	0.0258	0.0013	0.065
1.0%	0.014	0.064	0.005	ND	0.29	ND	0.0003	0.0003	0.003
1.8%	0.038	0.128	0.011	ND	0.45	ND	0.0004	0.0003	0.003
3.2%	0.071	0.190	0.018	ND	0.83	ND	0.0007	0.0004	0.005
End 72-h Static Renewal									
1.0%	ND	0.015	ND	ND	0.42	ND	0.0004	0.0003	0.002
1.8%	0.013	0.037	ND	ND	0.67	ND	0.0006	0.0002	0.005
3.2%	0.029	0.088	0.009	ND	0.72	ND	0.0007	0.0004	0.005
Start 4th 24-h Static Renewal									
100% Stock	2.166	7.579	1.169	ND	24.95	0.015	0.0240	0.0014	0.071
1.0%	0.016	0.052	0.008	ND	0.30	ND	0.0003	0.0004	0.002
1.8%	0.037	0.124	0.018	ND	0.47	ND	0.0005	ND	0.004
3.2%	0.075	0.204	0.037	ND	0.77	ND	0.0008	0.0004	0.006
End 96-h Static Renewal									
1.0%	0.007	0.024	ND	ND	0.34	ND	0.0002	ND	0.002
1.8%	0.014	0.047	0.005	ND	0.58	ND	0.0005	0.0006	0.003
3.2%	0.030	0.104	0.013	ND	0.77	ND	0.0008	0.0008	0.005
End 96-h Static[c]									
1.0%	0.0012	0.003	ND	ND	0.58	ND	0.0006	0.0007	0.003
1.8%	0.0019	0.007	ND	ND	0.63	ND	d	0.0004	0.004
3.2%	0.0036	0.014	0.006	ND	0.88	ND	0.0009	0.0007	0.004

[a]ND = non-detectable (<0.005 mg/L-C_2Cl_6, CCl_4 and C_6Cl_6; <0.002 mg/L-Pb; <0.0002 mg/L-As).

[b]All organisms dead (5.6 and 10.0%). No samples analyzed.

[c]Lower detection limits achieved by injecting 10 µL samples.

[d]Sample lost.

A-4. CONCENTRATIONS OF DISSOLVED COMPONENTS OF SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE DURING *Pimephales promelas*, *Hexagenia bilinata*, *Ictalurus punctatus*, *Lepomis macrochirus*, *Gammarus pseudolimnaeus*, and *Selenastrum capricornutum* TESTS

Organism	Dilution[a]	Component Concentration (mg/L)								
		CCl_4	C_2Cl_4	C_2Cl_6	C_6Cl_6	Zn	Pb	Cd	As	Al
Fathead minnow	Stock-SRO	2.09	6.87	1.26	ND	21.35	0.003	0.0225	0.0008	0.034
	Stock-SR1	2.33	7.39	1.50	ND	23.75	0.005	0.0236	0.0012	0.030
	Stock-SR2	2.16	6.98	0.79	ND	23.35	0.004	0.0215	0.0011	0.039
	Stock-SR3	2.28	7.17	1.45	ND	23.30	0.003	0.0227	0.0010	0.035
Mayfly larva	Stock-SR1	2.09	6.87	1.26	ND	21.35	0.003	0.0225	0.0008	0.034
	Stock-SR2	2.33	7.39	1.50	ND	23.75	0.005	0.0236	0.0012	0.030
Channel catfish	Stock-S	0.82	2.84	0.33	ND	20.30	0.002	0.0244	0.0012	0.060
	5.6%	0.02	0.06	0.01	ND	1.21	ND	0.0014	ND	0.007
	10.0%	0.05	0.13	0.02	ND	1.93	ND	0.0024	ND	0.010
	18.0%	0.09	0.34	0.05	ND	3.34	ND	0.0046	ND	0.015
	32.0%	0.17	0.41	0.10	ND	6.70	0.001	0.0084	0.0005	0.019
	56.0%	0.34	0.75	0.13	ND	10.60	0.001	0.0144	0.0009	0.033
Bluegill and Amphipod	Stock-S	0.75	3.36	0.72	ND	23.60	0.003	0.0332	0.0015	0.047
	5.6%	0.02	0.07	0.02	ND	1.33	ND	0.0018	ND	0.003
	10.0%	0.05	0.23	0.05	ND	2.32	ND	0.0036	ND	0.005
	18.0%	0.09	0.45	0.11	ND	4.22	ND	0.0066	ND	0.007
	32.0%	0.10	0.47	0.15	ND	7.06	0.001	0.0108	0.0009	0.016
	56.0%	0.26	0.90	0.24	ND	13.50	0.002	0.0157	0.0010	0.027
Alga	Stock-S	0.72	2.48	0.22	ND	28.00	0.024	0.0232	0.0006	0.069
	5.6%	0.02	0.06	0.01	ND	2.22	0.004	0.0029	ND	0.006
	10%	0.05	0.12	0.02	ND	3.00	0.005	0.0041	ND	0.010
	18%	0.08	0.31	0.03	ND	5.60	0.006	0.0052	ND	0.018
	32%	0.08	0.32	0.09	ND	8.90	0.010	0.0080	0.0003	0.031
	56%	0.29	0.72	0.11	ND	14.90	0.015	0.0132	0.0004	0.041

[a]Stock = 100% stock solution; S = static; SR# = static renewal number.

A-5. CONCENTRATIONS OF DISSOLVED COMPONENTS IN STOCK SOLUTIONS OF THE SYNTHETIC-HC SMOKE COMBUSTION PRODUCTS MIXTURE USED DURING 48-H TOXICITY TESTS TO DETERMINE SEPARATE COMPONENT TOXICITY TO *Daphnia magna*. STATIC SYSTEM WITH TESTS CONDUCTED AT 22°C

Test	Date	Dilution	Component Concentration (mg/L)								
			CCl	C_2Cl_4	C_2Cl_6	C_6Cl_6	Zn	Pb	Cd	As	Al
Total Mix	10/21/86	100% Stock	0.81	3.54	0.33	ND	22.00	0.006	0.0340	ND	0.050
		32%	0.13	0.55	0.10	ND	6.30	0.002	0.0117	ND	0.018
		18%	0.05	0.24	0.03	ND	3.40	0.001	0.0071	ND	0.010
		10%	0.04	0.16	0.02	ND	2.05	ND	0.0041	ND	0.003
		5.6%	0.02	0.09	0.01	ND	1.37	ND	0.0021	ND	0.002
		3.2%	0.01	0.03	0.01	ND	0.72	ND	0.0013	ND	ND
Zinc Only	9/25/86	100% Stock	ND	ND	ND	ND	22.60	ND	0.0003	ND	0.006
		56%	-[a]	-	-	-	15.20	-	-	-	0.003
		32%	-	-	-	-	7.90	-	-	-	ND
		18%	-	-	-	-	4.50	-	-	-	ND
		10%	-	-	-	-	2.72	-	-	-	ND
		5.6%	-	-	-	-	1.48	-	-	-	ND
Zinc Only	10/21/86	100% Stock	-	-	-	-	23.20	ND	ND	ND	0.003
		32%	-	-	-	-	7.10	-	-	-	ND
		18%	-	-	-	-	4.30	-	-	-	ND
		10%	-	-	-	-	2.90	-	-	-	ND
		5.6%	-	-	-	-	1.51	-	-	-	ND
		3.2%	-	-	-	-	0.78	-	-	-	ND
Metals Only	9/24/86	100% Stock	ND	ND	ND	ND	23.70	0.007	0.0434	0.0007	0.054
		56%	-	-	-	-	15.30	0.004	0.0229	0.0004	0.034
		32%	-	-	-	-	7.90	0.002	0.0126	0.0003	0.019
		18%	-	-	-	-	4.50	0.002	0.0065	ND	0.009
		10%	-	-	-	-	2.70	0.001	0.0037	ND	0.005
		5.6%	-	-	-	-	1.45	ND	0.0023	ND	0.003

(Continued next page)

A-5. (CONTINUED)

Test	Date	Dilution	Component Concentration (mg/L)								
			CCl_4	C_2Cl_4	C_2Cl_6	C_6Cl_6	Zn	Pb	Cd	As	Al
Total	9/25/86	100% Stock	0.69	3.18	0.97	ND	0.121	0.016	0.0257	ND	0.077
less		56%	0.16	0.70	0.28	ND	0.061	0.010	0.0141	ND	0.045
Zinc		32%	0.07	0.35	0.16	ND	ND	0.005	0.0074	ND	0.028
		18%	0.07	0.34	0.12	ND	ND	0.003	0.0047	ND	0.015
		10%	0.03	0.10	0.05	ND	ND	0.002	0.0025	ND	0.008
		5.6%	0.02	0.07	0.03	ND	ND	ND	0.0011	ND	0.005
Organics	9/24/86	100% Stock	0.55	2.32	0.78	ND	0.130	ND	0.0006	ND	ND
Only		56%	0.18	0.68	0.25	ND	-	-	-	-	-
		32%	0.09	0.35	0.15	ND	-	-	-	-	-
		18%	0.06	0.28	0.10	ND	-	-	-	-	-
		10%	0.03	0.11	0.05	ND	-	-	-	-	-
		Control	ND	ND	ND	ND	ND	ND	0.0005	ND	0.003

[a]Samples not analyzed.

APPENDIX B

ADDITIONAL GOOD LABORATORY PRACTICE STANDARDS REPORT REQUIREMENTS

U.S. EPA Good Laboratory Practice (GLP) Standards (EPA 1983. Fed. Reg. 48:53946-53969) for non-clinical studies require the following additional statements be included in all final reports which summarize data collected under GLP Standards:

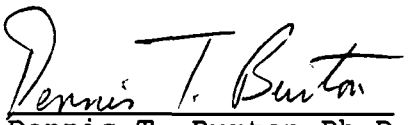
Study director: Dennis T. Burton, Ph.D.

Project scientists: Daniel J. Fisher, Ph.D
Robert L. Paulson, M.S.

Location of all raw data, documentation, records, data reports, Quality Assurance Unit (QUA) reports, and final report:

Archives
The Johns Hopkins University
Applied Physics Laboratory
Environmental Sciences Group
Shady Side, MD 20764

Final Quality Assurance Unit statement: Page 74 of this report



Dennis T. Burton,Ph.D.
Study Director

Date: 1/5/87

MEMORANDUM

To: Dr. Dennis T. Burton
Study Director

From: Lenwood W. Hall, Jr. - QA/QC Officer

Date: January 20, 1987

Subject: Final Good Laboratory Practice (GLP) Report, Army Project MIPR 85 MM 5505

I have reviewed the draft of the final report for Phase II of the project submitted to me on January 16, 1987. The report accurately describes the methods and standard operating procedures originally submitted for the project and accurately reflects the raw data.

I conducted quarterly QA/QC inspections during Phase II of the project. These inspections were conducted on February 25, May 28, August 26, and October 23, 1986. The project completion deadline was extended to January, 1987 with the inclusion of extra studies requested by the sponsor and completed at no additional charge to the contract. The study was completed on schedule according to the revised schedule. My inspections indicated that the intent of the GLP regulations were followed, although a number of minor problems were reported. These problems were corrected to my satisfaction.

Lenwood W. Hall, Jr.

Lenwood W. Hall, Jr.

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